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BUNODERA CORNUTA SP. NOV.: A NEW PARASITE FROM THE CRAYFISH AND CERTAIN FISHES OF LAKE CHAUTAUQUA, N. Y.

HENRY LESLIE OSBORN.

A trematode is met frequently at Chautauqua, New York, which though already known seems never to have been critically studied and described. While generically identical with *B. nodulosa* Zeder, of Europe, it cannot be referred to the same species. A form mentioned by Kellicott, '83, and Wright, '84, and Linton, '92, may be identical with it. I have not yet had access to the articles of the first two writers, but Linton, '92, regards the form they mention as identical with the one which he describes from cysts from the ovary of crayfish from Alma, Michigan, which, while it is much like *B. nodulosa* of Europe, he regards as distinct, on account of the two lateral papillary appendages projecting from the oral sucker and of triangular shape. Ward, '94, reports at Ann Arbor, Mich., the form mentioned by Kellicott, Wright and Linton, and considers it probably identical with *B. nodulosa* of Europe. I am at present inclined to think that of these four cases at least that of Linton is identical with the Chautauqua form, and that the others may be. My knowledge of *B. nodulosa* is almost entirely drawn from the account of it in Looss' ('94) admirable monograph of the fish and frog distomes, as I have not had access to specimens of that form. A related trematode is described and figured by Linton, '98, from the intestine of the lake sturgeon, and referred to *B. auriculata* of Wedl, '57. A single specimen of the material on which Linton's account was based has been loaned me by the U. S. National Museum through the kindness of Dr. C. W. Stiles, and from such examination of it as I could make without injuring it I was able to see that externally it is essentially like *B.*

cornuta, excepting as regards the oral papillæ. On this point and in the figures of Linton, there is a divergence from either *B. nodulosa*, for the ventral papillæ are transverse and in the form of a horn, and from the Chautauqua form for the four anterior papillæ characteristic of both *nodulosa* and *cornuta* are wholly wanting. If the absence of these papillæ is a constant character, as at present it must be assumed to be, we then must accept three species for this genus. The coarser features of the organization of the Chautauqua form is described in the following

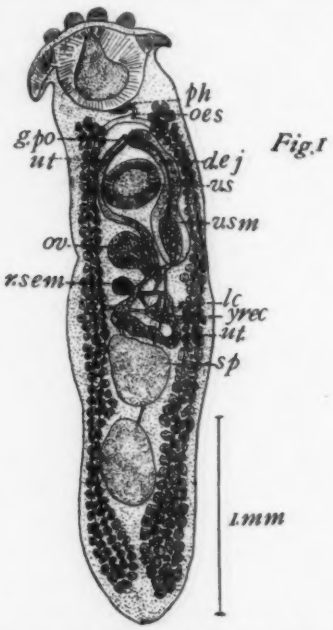

Fig. 1

FIG. 1. *B. cornuta*, ventral view, compressed, camera lucida × 30. *d.e.j.*, ductus ejaculatorius; *g.po.*, genital pore; *l.c.*, Laurer's canal; *oes.*, œsophagus; *ov.*, ovary; *ph.*, pharynx; *r.sem.*, seminal receptacle; *sp.*, spermary; *ut.*, uterus; *v.s.*, ventral sucker; *v.sem.*, seminal vesicle; *y.rec.*, yolk receptacle.

pages. An account of some points in the minuter structure is reserved for a later article. It will be necessary and convenient at least till more is known of *B. auriculata* to adopt a name for the Chautauqua species, and I propose for it the name *B. cornuta*.

The adult stage of *B. cornuta* is found at Chautauqua in the stomachs of black-bass, rock-bass and cat-fish or bull-heads, caught near the Assembly grounds, and earlier stages are found encysted in crayfish, caught near the shore just above the grounds. These localities are near the head of the lake. I have not explored the lake in other places and cannot say how generally the fluke is found in it. The crayfish is clearly the host immediately prior to the fish, as partly digested crayfish are present in the stomachs of fishes where the cysts and the young just escaped flukes are found. The infection of the crayfish is prac-

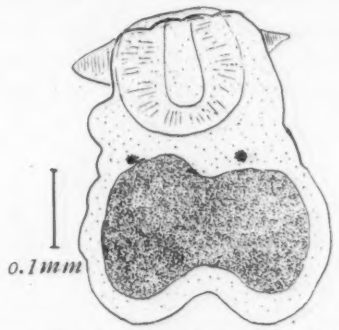

FIG. 2. Young worm still enclosed in cyst, × 120, the shaded area was opaque, and white by reflected light.

tically universal. The flukes are always found encysted, never free. They are located in the parts immediately related to the reproductive system, most constantly in the muscles, especially those running from the thorax to the abdomen, also in the heart itself, and in the gonads. Remoter organs are not infected. This mode of occurrence indicates that the infection may be through the ducts of the gonads, but I have no observations to decide this point. The number of cysts per individual varies considerably, in one case 40 were found, distributed as follows: 25 in the muscles, 6 in the walls of the heart, 9 in the spermary; in another case: 16 in the muscles, none in the heart or gonad; in still another a few were seen in the muscles and none in the heart or gonads. The cysts vary somewhat in size and structure with the season. In early July they are 0.9 mm. in diameter and consist of a soft fleshy grayish enveloping portion about 0.2

mm. thick, enclosing a central mass, dark yellowish-brown and hard, as if perhaps chitinous, of a diameter of 0.5 mm. By manipulating the cysts with little knives made from specially ground needles I found it possible to extract from them a very immature fluke (see Fig. 2) recognizable as *B. cornuta* by its oral sucker. A pair of eyes is present, but the inner organization showed no traces. I suppose the dark granular mass at the posterior end to be a supply of food for the developing worm. Some of the cysts differ by having in place of the hard grandular inner cyst a thin homogeneous covering, enclosing a worm so

FIG. 3. Sagittal section, cam. luc. × 60. *cir.*, cirrhus; *ex.bl.*, excretory bladder; *o.p.*, oral papilla; *pph.*, pre-pharynx.

much more advanced in development, that the alimentary and excretory systems were formed and the genital organs well advanced. In early August cysts having a diameter of 1 mm. or over were found which contained fully matured worms, containing embryos numerous enough to impart a distinctly brown tinge to the parent. These facts are of very considerable interest,

for they indicate that the young worm develops actively during encystment, and that here self-fertilization must take place. A fuller study of this point is desirable.

In the fish the parasite has been found only in the stomach. Both cysts separated from the crayfish, and the free worms are found. *B. nodulosa* is reported from the intestine of fishes and *B. auriculata* is also an intestinal parasite.

The body form is nearly cylindrical, in contrast with the elongate neck and almost leaf-shaped body of *B. nodulosa*. This contrast is well seen by comparing Figs. 1 or 7 with Fig. 10 of Looss. The latter is a young stage in which the vitellaria are not as yet developed, while both of the Chautauqua specimens possess them and the uterus contains eggs. My specimens differ considerably in length, owing to the fact that they go on growing

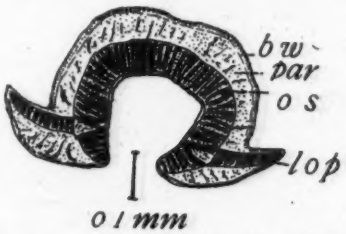

FIG. 4. Transverse section passing through the oral sucker in the level of the lateral processes. Cam. luc. × 50. *l.o.p.*, lateral oral papilla; *b.w.*, body wall, *par.*, parenchyma; *o.s.*, oral sucker.

longer after maturity. The longest one that I have seen measured 3.0 mm. in length by 0.9 mm. in width (in the preserved and mounted state). I have seen specimens fully developed sexually measuring only 0.9 mm. in length by 0.2 mm. in width. The oral sucker is very large, so that it fills completely the anterior end of the body. It is furnished with remarkable muscular processes, six in number which give the worm a very characteristic appearance. Four of these processes or papillæ are blunt, and extend forward from the dorsal and anterior end of the body. In a ventral view of the animal they are seen extending slightly beyond a thin layer of the body wall which forms the anterior boundary of the body. The other two papillæ are at the posterior level of the oral sucker, and ventral, on the opposite

side from the four blunt anterior papillæ, and they are extended transversely to the animal. In form they are tapering and pointed, and slightly curved backward, in the form of a horn, extending considerably beyond the contour of the side of the animal. The oral sucker itself has a diameter of 0.4 mm. The ventral sucker, while large, is smaller than the oral sucker, its diameter being 0.3 mm. Its position in Fig. 1 is strikingly far forward; in Fig. 7 it is more nearly in the center of the body. This difference is due to contraction of the neck in Fig. 1, shown also by the winding course of the œsophagus of that specimen. The genital pore is located in front of and near to the ventral sucker, in the middle line. Eyes are present in younger specimens, but older ones do not possess them, though in these it is

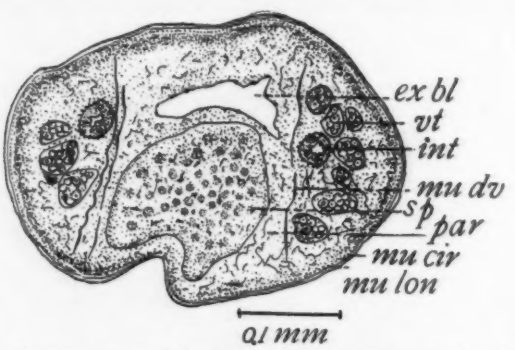

FIG. 5. Transverse section in level of the posterior spermary. Cam. luc. × 115. *vt.*, vitellaria; *mu.d.v.*, dorsiventral muscle; *mu.cir.*, circular muscle of the body wall; *mu.lon.*, longitudinal muscle of the body wall.

possible to find scattered grains of pigment in the region of the pharynx, indicating their late disappearance. Looss represents eyes in both the specimens figured by him, so that if they are not exceptional cases, the eyes persist to a much later time in the life history of *B. nodulosa* than of *B. cornuta*.

The body wall presents the usual cuticle, destitute of spines. The usual muscle layers are present, the fibers of the outer circular layer are very fine indeed; those of the longitudinal and oblique layers are exceptionally large. Parenchymatous muscle is somewhat specially collected in each side of the body running dorso-

ventrally, and marking off a lateral area, containing the vitellaria and the intestine, from the center (see Fig. 5). There are no horizontal parenchyma muscles. Cells of the parenchyma directly underlying the body wall are especially numerous and glandular in appearance, as often in trematodes.

The oral sucker opens widely downwards and forwards. It is composed of the usual muscular masses, enclosed within a fine structureless membrane marking it off from the parenchyma. The detailed structure of the papillæ is indicated in Fig. 4, which is a camera lucida drawing from a section passing through the

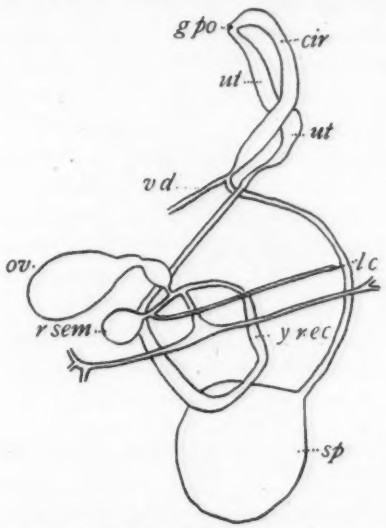

FIG. 6. General view of the reproductive organs, seen dorsally.

oral sucker in the level of the lateral papillæ and through their length. Sections through the four anterior papillæ show the same things. The wall of the oral sucker, consisting of a layer of parenchymatous tissue and masses of radial fibers, is pushed out at the bases of the papillæ, the connective tissue portion being directly continuous and the muscle fibers pushed aside at that point, and passes up to the summit of the papilla, a new set of radial fibers being added in the papilla similar to those of the general wall of the sucker. The papillæ are thus not merely

surface features of the animal, but deep-seated in their origin, and are entitled to be regarded as of considerable importance from a taxonomic point of view.

The lateral papillæ are unmistakable organs not likely to be overlooked by an observer, whereas the ventral papillæ of *B. nodulosa* are inconspicuous and might easily escape notice, a point discussed by Looss ('94, p. 34). In *B. auriculata* I looked very closely for the dorsal papillæ without finding them and I am

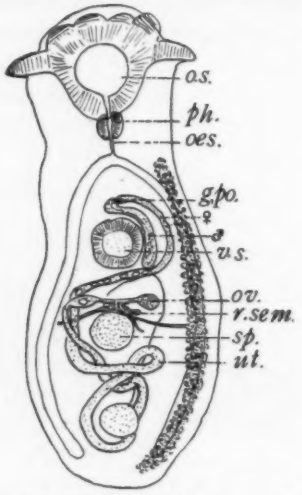

FIG. 7. Ventral view of a specimen in which the uterus is most fully developed, the vitellaria are omitted from the right side.

convinced that they are absent from the specimen I saw, in which respect my observations confirm those of Linton as indicated in his figures, '98, Pl. XLV., Figs. 1–7.

There is a short pre-pharynx, a small pharynx, about 0.05 mm. long, a short œsophagus, its walls very strongly muscular and surrounded by glandular cells. The forking of the intestines is thus close to the pharynx, a point different from *B. nodulosa*. The intestines are simple and long, reaching to near the hinder end of the body. They are lined with epithelium cells whose outer ends are elongate and whose tips extend into the cavity of the organ. Circular and longitudinal muscle fibers are present in the wall.

The excretory pore is terminal. Close in front of it is the excretory bladder, which in sections can be seen running dorsally forward at least as far as the level of the front of the anterior testis (see Figs. 2, 5). I have not been able to recognize more than the most posterior portion in living animals. According to Looss there is in *B. nodulosa* a bladder wholly posterior to the hinder testis containing concretions, and from which vessels run forward on either side. I have not seen such concretions in *B. cornuta* and the bladder is much more extensive than that.

There are two large testes, 0.3 mm. across, lying one directly in front of the other. In *B. nodulosa* the testes are smaller, farther apart and oblique. The testes are crowded with active sperm cells, many of them in the last stages of spermatogenesis, and with numerous fully formed spermatozoa. The seminal vesicle and spermatic receptacle are also filled with them.

Long and slender vasa deferentia run dorsally to the other genital organs, and meet at the posterior end of the large cirrhus sack which is located some distance behind the posterior border of the ventral sucker. The cirrhus sack is very large indeed, much larger than in *B. nodulosa*. It has a definite outer wall, strongly muscular, enclosing a tubular passage subdivided into two portions, a posterior thin-walled part, the seminal vesicle, and an anterior ductus ejaculatorius. This latter is surronded by glandular "prostate" cells, is very strongly muscular, having both circular and longitudinal fibers. The ductus ejaculatorius is not coiled. I do not know whether it is eversible or not.

The ovary is generally located on the right side, but not infrequently it is found on the left (cf. Figs. 1 and 7). It is always near the ventral sucker, a large and conspicuous organ. There is a short ciliated oviduct, soon joined by first a duct from the seminal receptacle, then one from the yolk receptacle. Certain glandular-looking cells which lie around the oviduct may perhaps represent a shell gland, but a distinct and well-marked organ is not present. Nearly all of my specimens appear to be quite young, and though the uterus contains eggs it is not fully developed. In one, however (Fig. 7), the uterus is longer and evidently more as in fully matured individuals. In this case the uterus is distinctly tubular and winds down and back, passing

between the testes in its course, in *B. nodulosa* the uterus is a large sack containing old and young eggs indiscriminately, the uterus is saccular even in young individuals of *B. nodulosa*, as seen in Looss' Fig. 10 of a specimen before egg production has begun. The terminal part of the uterus differs decidedly from the rest so as to form an entirely distinct though continuous organ (cf. Fig. 2.) Its wall is very thick indeed and consists of a strong muscular coat quite unlike the wall of the deeper parts of the tube, and within the wall is furnished with a clothing of very peculiar numerous long slender bluntly ending processes which are free at tip in the cavity of the organ. These structures do not look like cilia, being too blunt. They do not seem certainly to be protoplasmic, at least the bases do not seem — as far as I have been able to study them — to be nucleated cells, as we should expect. The histological structure of this part will have to be left for a subsequent study. This organ is further surrounded by parenchyma cells having much the same appearance as the prostate cells of the cirrhus. The eggs measure 0.07 mm. in length instead of 0.1 mm. as in *B. nodulosa*.

Laurer's canal is present, passes dorsally and opens to the exterior on the left side. The seminal receptacle is large and distinct; it lies close to and just behind the ovary. It is in all cases of adults filled with spermatozoa. The vitellaria are large, and located as above described laterally and so as partly to envelope the intestines. They extend from near the phaynx to near the hind end of the body and consist of very numerous small follicles uniformly distributed. A duct from each crosses the body in front of the anterior testis and behind the ovary and seminal receptacle and the two joining from the yolk receptacle which reaches the oviduct by a short duct close to the ovary.

The points made in the foregoing pages are summarized in the following table of comparisons:

	B. cornuta.	*B. nodulosa.*
Total length,	3 mm.,	3 mm. Looss, 4.5 mm. Olsson.
Body form,	cylindrical,	leaf-shaped.
Neck,	not prominent,	prominent and distinct.

Lateral papillæ,	transverse and hook-shaped,	longitudinal and blunt, not hook-shaped.
Eyes,	not persistent,	persistent in adult.
Œsophagus,	short,	long.
Excretory bladder,	long,	short.
Testes,	close together in median line,	wide apart, and oblique.
Uterus,	tubular,	saccular.
Ova,	0.07 mm. long,	0.1 mm. long,
Residing,	in stomach,	in intestine of host.

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Saint Paul, Minn., March 10, 1903.

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ON THE BLOOD VESSELS, THEIR VALVES AND THE COURSE OF THE BLOOD IN LUMBRICUS.[1]

J. B. JOHNSTON.

In a previous paper[2] an account has been given of the experimental study of the course of the blood flow in *Lumbricus* The most important result there set forth was that the circulation is not segmental but strictly systemic. The course of the flow is as follows: forward in the dorsal vessel for its whole length; downward in the hearts; both forward and backward from the hearts in the ventral vessel; outward from the ventral to the body wall, nephridia and intestinal wall; toward the lateral neurals from the body wall; backward in the subneural; upward to the dorsal vessel in the parietals from the subneural, the nephridia, and the body wall, and in the dorso-intestinals from the intestine. Thus, there is no circuit of blood in each segment to which a sytemic circuit for part of the blood has been superadded, as all previous authors have maintained, but all of the blood flows in a single systemic circuit. In the head region the blood is carried forward beyond the hearts by both dorsal and ventral vessels and is returned to the dorsal behind the hearts in larger part by the lateral œsophageals, and in smaller part by the subneural and the parietals of XII. and succeeding somites. The lateral œsophageal system is considered to represent the parietals in the somites anterior to XII.

This view of the circulation raised two important questions which further work has answered: (1) What happens when the hearts are removed from the circulation by decapitating the worm? Do the conditions which obtain in the regenerating

[1] Studies from the Zoölogical Laboratory of West Virginia University, No. 8, February 28, 1903. A part of the work reported here was done by my former student, Miss S. W. Johnson. For the conclusions reached the present writer alone is responsible.

[2] "The Course of the Blood Flow in *Lumbricus*," by J. B. Johnston and Sarah W. Johnson, *Amer. Naturalist*, April, 1902.

worm confirm the above results? (2) What is there in the structure of the blood vessels to determine and control the course of the blood?

The first question has been answered by a series of regeneration experiments carried out upon large and small specimens of *Lumbricus*. Operations removing from eleven to twenty somites from the anterior end were performed upon 171 worms. These were examined alive from time to time and eventually 20 were hardened for sectioning. The time that the worms were allowed to live varied from ten days to three and a half months. In a few worms regeneration progressed well, but the majority died after a few days or weeks. A detailed report upon these experiments would not be profitable for our present object. Although there were very great variations in the condition of the blood vessels, the following may be said to be true in greater or less degree of all the worms studied alive or sectioned. The vessels in the anterior one fourth to one half of the worm were greatly crowded and distended with blood. The anterior portion of the worm was usually a bright red to the naked eye and under a lens many small vessels not usually visible were distinctly seen. Sections showed that all the vessels were more or less crowded with blood, while the dorsal, subneural, and the vascular plexus of the intestinal wall showed the greatest distension. The ventral vessel was seldom stretched much beyond its normal size, while the subneural was often as great in diameter as the ventral. Occasionally the subneural was much larger than the ventral and sometimes its cross-section was equal to that of the nerve cord. In several cases the vascular layer of the intestine was very greatly crowded and, considering its great capacity in normal conditions, it is probable that it always held the greatest accumulation of blood. The posterior portion was very poor in blood in all worms.

These conditions are readily explained in accordance with the scheme of circulation above summarized. The fulness of the dorsal, intestinal and subneural vessels is due to the pressure from the dorsal which is deprived of the normal outlet for the blood carried by it, and forces the blood downward in the dorso-intestinals and parietals contrary to its usual course. The small

amount of blood in the ventral is due to the absence of the hearts and the inability of the dorsal to drive the blood through the capillary systems to the ventral. The absence of blood in the posterior end is a further result of the small amount of blood received by the ventral. If there were a segmental circulation in *Lumbricus* there would probably be no great accumulation of

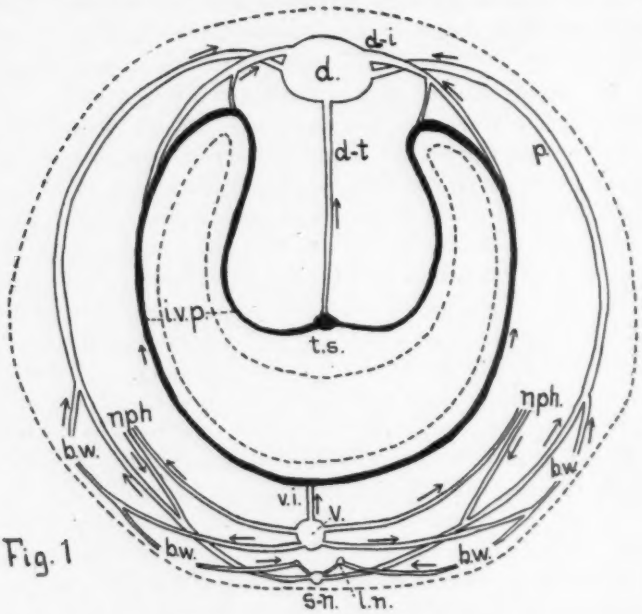

Fig. 1

FIG. 1. General scheme of circulation in body region, all the vessels of one segment being projected upon the plane of a transverse section. The vascular layer of the intestine is shown by a broad black line. In the typhlosole the vascular plexus thickens at one place to form the typhlosolar sinus which varies greatly in size in different worms and in different parts of the same worm. From this sinus three dorso-typhlosolar vessels in each segment carry blood to the dorsal vessel. These vessels and the branching of the dorso-intestinal vessels shown in this figure have not before been correctly described or figured for *Lumbricus*.

blood at the anterior end in these experiments, since the segmental circulation would tend to relieve the systemic and the even distribution of the blood would be maintained in accordance with the law of least resistance. These regeneration experiments,

therefore, seem to confirm the results of physiological experimentation. No effort was made to trace the development of hearts in the regenerated heads and the final reorganization of the circulation, and it is doubtful whether the worms would have lived long enough for this purpose.

It is probable that the failure of the blood vessels to adjust themselves to the new conditions is at least one of the chief causes of the death of worms under these experiments. The continued strong pulsations of the dorsal vessel after the removal of the hearts force the blood out through vessels which normally empty into it. In some cases the reversal of flow through the vessels of the body wall and intestines is produced readily enough to allow the worm to survive the operation, but in most cases less blood would reach the ventral vessel than is necessary to supply the posterior end of the worm and an insufficient amount of blood would pass through the respiratory plexus beneath the hypodermis. The blood which leaves the dorsal vessel in the anterior part of the worm either settles in the vascular layer of the intestine, which readily expands to receive it, or passes directly through the parietals to the subneural, which is consequently greatly expanded; and avoids the respiratory plexus because of the resistance in that quarter. A similar withdrawal of blood from the respiratory plexus of the posterior end of the worm also results indirectly from the small amount of blood in that region, so that the whole worm is seriously deprived of needed oxygen. In the normal circulation the blood is driven to the respiratory plexus from the ventral under direct pressure from the hearts, and there is no other way of less resistance by which the blood may return from the ventral to the dorsal. Upon the view of the circulation held by Bourne[1] and Harrington,[2] namely, that the dorso-intestinals empty into the dorsal vessel and the parietals carry blood away from it, it is evident that the path of least resistance from the pulsating dorsal vessel is through the parietals directly to the subneural and that there would be nothing to drive the blood through the respiratory

[1] Bourne, A. C., "On *Megascolex cæruleus* and a Theory of the Course of the Blood in Earthworms," *Q. J. M. S.*, Vol. 32, p. 49, 1891.

[2] Harrington, N. R., "The Calciferous Glands of the Earthworm, with an Appendix on the Circulation," *Jour. Morph.*, Vol. 15, Suppl., p. 105, 1899.

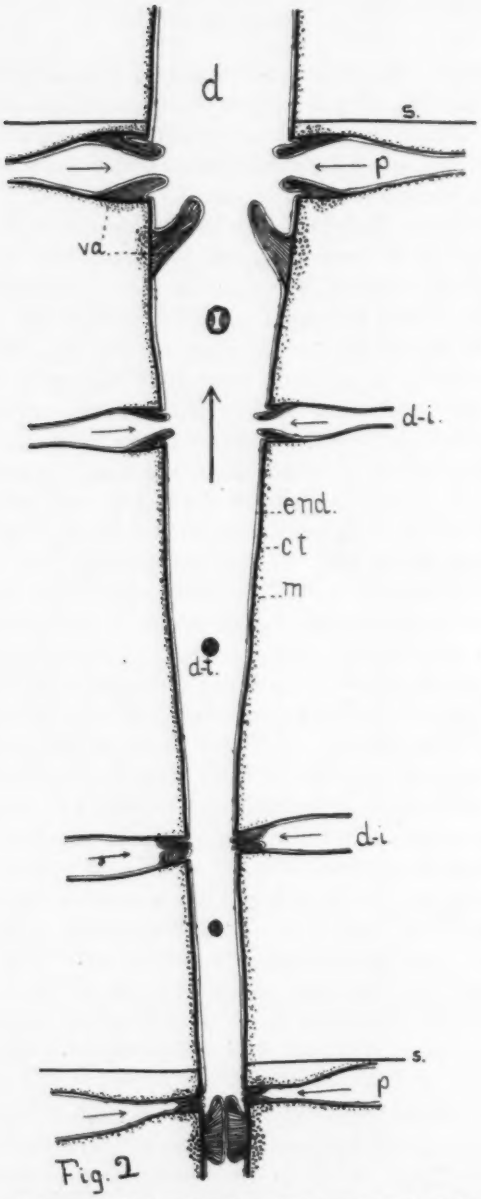

FIG. 2. A diagrammatic horizontal section of the dorsal vessel and those emptying into it. One somite and part of a second are shown, and the last part of a pulse wave and the greater part of a following contraction wave are represented. The arrows show the course of the blood and the position and changes of form of the valves are shown. The chloragogue cells covering the walls of the vessels are not drawn.

capillaries of the body wall. This is perhaps an insuperable objection to that theory of the circulation. This objection does hold against the view of Perrier[1] and Benham,[2] according to which the blood flows to the dorsal vessel in the parietals and out from it in the dorso-intestinals.

The study of the structure of the vessels shows that the movement of the blood is determined by the structure of the walls and by definite valves which several of the vessels possess. The wall of the dorsal vessel consists (Fig. 2) of a lining endothelium of very thin cells whose nuclei alone are usually visible; a connective tissue layer containing a few longitudinal (muscle?) fibers, and a well-developed layer of circular muscle fibers. Outside these are the chloragogue cells. To the layer of circular muscle fibers are due the pulsations of the dorsal vessel, and thickenings of this layer at certain points assist in the action of the valves, as will be described below. The wall of the ventral vessel has no circular muscle layer. Its lining endothelium is more conspicuous than that of the dorsal vessel and the connective tissue layer is very thick. This is a strong fibrous layer and gives great rigidity to the wall of the ventral vessel. Outside of the connective tissue layer are a few (4 to 6) strands of longitudinal fibers which take the same stain as the muscle fibers in the sheath of the neighboring nerve cord. Outside these fibers is a layer of peritoneum closely similar to that covering the inner surface of the body wall.

The subneural consists of only the endothelium and connective tissue layer, outside of which is the sheath of the nerve cord. This is the structure of the lateral neurals also, and of all the smaller vessels. The dorso-intestinals and parietals present an intermediate condition between those with and those without a circular muscle layer. The dorso-intestinal vessels are devoid of muscle fibers except at their dorsal ends where there is a thin extension of the circular layer of the dorsal vessel for a short distance. The parietals are provided with a thick band of circular fibres close to their connections with the dorsal and the layer is

[1] Perrier, Edw., "Recherches pour servir à l'histoire des Lombriciens terrestres," *Nouv. Arch. du Mus. d' Hist. Nat.*, Paris, Tome 8, 1872.

[2] Benham, W. B., "The Nephridium of *Lumbricus* and its Blood Supply," *Q. J. M. S.*, Vol. 32, p. 293, 1891.

continued along the vessel for about a third or half its length. These muscle fibers in the dorsal portion of the parietals produce the active pulsations which have been described in an earlier paper (*loc. cit.*, p. 323).

The walls of the hearts have the same structure as that of the dorsal vessel, except that they are covered with chloragogue cells only in their dorsal portion, elsewhere by peritoneum. The circular muscle fibers are large and the layer somewhat stronger than that in the dorsal vessel.

The structure of the vessels determines whether they shall propel the blood by their pulsations or only carry it, and the account of the structure accords with the well-known facts concerning the pulsations of the vessels. Pulsations in the dorsal, parietals and hearts are well established; pulsations in other vessels, described by Harrington, have not been seen by the author and to whatever extent they occur they must be produced without muscle fibers.

Valves are present in the dorsal vessel and in all the vessels connected with it, namely, the dorso-intestinals, dorso-typhlosolars, parietals, lateral œsophageals (?) and hearts. The valves in the dorsal are a pair of large thick flaps attached to the lateral walls of the vessel at a point a short distance behind each septum and immediately behind the openings of the parietals. These valves are always directed forward and allow the free passage of blood during the pulse wave. As the contraction wave approaches, the valves are brought into contact and at the moment of greatest constriction the two flaps are tightly pressed together and completely close the lumen of the vessel. The efficiency of the valves is secured and increased by a considerable thickening of the circular muscle layer at the valve (Fig. 2). The valves do not act in the ordinary manner of flap valves, but the two fleshy flaps are pressed together and form a large mass which fills the vessel. In the region of the hearts a pair of valves is found in the dorsal vessel a short distance in front of each pair of hearts.

The valves in the dorso-intestinal, dorso-typhlosolar and parietal vessels are essentially the same in form and position. In each of these vessels (Fig. 2) a pair of small fleshy flaps are sit-

uated at the opening of the vessel into the dorsal. In the dorso-intestinal and parietal vessels the flaps are attached one to the anterior and one to the posterior wall of the vessel, and the body of the flap projects into the lumen of the dorsal vessel. In the dorso-typhlosolars the flaps are lateral in position, are situated deeper in the vessels, and do not project so far into the dorsal. It is evident that pressure from the dorsal toward any of these vessels would tend to close the valves. The closing of the parietals is further secured by a thickening of the circular muscle layer as in the dorsal; and in the dorso-intestinals a thin extension of the muscle layer of the dorsal serves the same purpose. Muscle fibers have not been observed in the dorso-typhlosolar vessels. The valves in these vessels allow the blood to flow from them into the dorsal only, and this accords with the results obtained by the earlier experimental investigation. In the case of the decapitated worms the valves in all these vessels near the anterior end must have been forced.

The hearts are better supplied with valves than are any of the other vessels. In each heart are four pairs of valves (Fig. 3); one situated close to the dorsal vessel, one between the first and second thirds from the dorsal end, one between the second and third thirds, and the fourth in the ventral end of the heart at the opening into the ventral vessel. The three pairs in the body of the heart are like those in the dorsal vessel but are smaller in proportion to the diameter of the heart. They are inclined downward and are large enough to close the heart during its contraction. The presence of these valves might seem unnecessary in view of the fact that the contraction waves pass along the heart from above downward. However, if from any cause the contraction becomes modified or irregular or if the whole heart contracts at once, the functional importance of these valves is evident. It is a matter of common observation that such irregularities in the contractions of both the hearts and the dorsal vessel do appear in worms dissected alive under an anæsthetic, and it is probable that such irregular contractions and the influence of movements of the body make necessary the valves in the hearts and the dorsal vessel in the normal worm. The valves in the smaller ventral ends of the hearts fill the lumen and project

into the ventral vessel very much as the valves in the parietals project into the dorsal. Thus, with the valves in the dorsal between each two pairs of hearts and the four valves in each heart, regurgitation of blood during the strong cardiac contractions is effectively guarded against.

The study of the fine structure of the valves has presented great difficulties because methods of fixation which give satisfac-

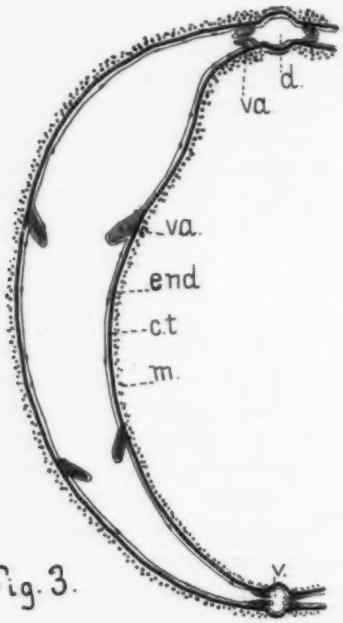

FIG. 3. A diagrammatic cross-section through one of the hearts to show the position of the valves. The chloragogue and peritoneal epithelium are not drawn.

tory preparations of all other tissues give very imperfect pictures of these valves. This itself indicates one fact regarding their structure, namely, that they are composed of very soft-bodied or watery cells which may appear vacuolated or shrunken, or even macerated. In many preparations the valves appear only as masses of granular or coagulated material containing many ovoid nuclei. In the most successful sections, however, the valves

show indistinct cell boundaries which produce an appearance of striations running from base to free edge of the valve. In most preparations, especially in longitudinal sections of the dorsal vessel, which are often oblique owing to the curves of the vessel, the substance of the valves appears to be sharply delimited from the connective tissue layer. This would indicate that the valve is formed by a thickening of the endothelial layer. It is difficult to disprove this first supposition because the endothelial cells are so broad that one can seldom expect to find an endothelial nucleus on the surface of a valve. However, in some cases in the hearts flattened nuclei similar to those of the endothelial cells are found on the surface of the valves. Cross-sections of the dorsal vessel through the base of the valves show a radial striation running from the valves through the connective tissue and muscle layers. From these facts it appears that the valves are composed of elongated cells which run through the connective tissue layer and securely anchor the valves. Since they are covered internally by endothelial cells they must be regarded as belonging to the connective tissue layer. Essentially the same structure is presented by all the valves, although those in different positions differ greatly in size and form in relation to the function which they have to perform. The largest valves are those in the dorsal vessel. These are thick flaps attached by broad bases to the lateral walls of the vessel. When the vessel is distended the valves are nearly semilunar in form. When the vessel is contracted the valves become greatly compressed against one another and the soft substance of the valve is forced both forward and backward from its point of attachment. When the valve extends far forward it overlaps the opening of the parietal vessel and might appear to function to close that vessel. Such a condition seems to have been seen by Benham (*loc. cit.*). The valves in the dorso-intestinal and parietal vessels are also paired flaps, but owing to the small size of the vessels the flaps are small at their bases and are longer than they are broad. Often these valves have a balloon form as they project into the dorsal vessel. The valves in the dorso-typhlosolar vessels are situated somewhat farther within the vessels and are more nearly simple semicircular flaps. The valves at the ventral ends of the hearts are relatively large

and project so far into the ventral vessel that they might be mistaken for valves proper to the ventral vessel itself.

The course of the blood flow is determined by the disposition of the valves as well as by the direction of the pulsations, and there is evidently entire agreement between the results of the physiological experiments and anatomical investigation. It is obvious that in small vessels or in such as receive blood from a capillary system so that there is no great pressure in the usual course, there may occur temporary reversals of flow due to movements of the body or other causes. Such reversals might most readily take place in the subneural vessel and such phenomena are probably the basis for Harrington's statement that the blood flows now forward, now backward in the subneural. However, the general course of the blood flow is strictly determined, as shown by the consistent experimental and anatomical results, and no considerable or long-continued reversal or interruption of the usual current are possible except as the result of violent interference such as decapitation of the worm.

The valves in the vessels have received very meager notices heretofore. The mention of valves in the dorsal vessel by Benham has been noticed above. A recent writer [1] has mentioned the presence within the dorsal vessel of cells similar to the chloragogue cells. These are also doubtless the valves of the dorsal vessel.

EXPLANATION OF FIGURES.

Abbreviations: *b.w.*, body wall; *c.t*, connective tissue layer of blood vessels; *d.*, dorsal vessel; *d-i.*, dorso-intestinal vessel; *d-t.*, dorso-typhlosolar vessel; *end.*, endothelial lining of vessels; *i.v.p.*, vascular plexus of intestine; *l.n.*, lateral neural vessel; *m.*, layer of circular muscle fibers in walls of vessels; *nph.*, nephridium; *p.*, parietal vessel; *s.*, septum; *s-n.*, subneural vessel; *t.s.*, typhlosolar sinus; *v.*, ventral vessel; *va.*, valve; *v.i.*, ventro-intestinal vessel.

[1] Rice, Wm. J., "Studies in Earthworm Chloragogue," BIOL. BULL., Vol. III., Nos. 1–2, 1902.

TWO NEW GENERA OF MALLOPHAGA.

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There have come to me recently specimens of Mallophaga, taken from birds from mid-ocean islands, which demand the founding of two new genera in this interesting but little-studied order of parasitic insects. In the order, as at present known, there are about 1,500 species, comprising twenty-three genera. The small number of genera is striking in itself, but is made more amazing when it is remembered that eleven of the genera comprise but thirty of the species, leaving thus nearly the whole bulk of the species included in the twelve remaining genera. The addition of two new genera is, therefore, rather notable in the development of our systematic knowledge of the group. Although about two hundred new species of Mallophaga have been described from North American birds but one new genus (my *Giebelia*, with only one species, from shearwaters) has had to be established, all the other North American species being referable to genera founded on Old World species and specimens. The following revised key to the known genera of the order (including the two new genera described in this paper) is presented for the use of beginning students of the group, or of general entomologists:

ANALYTICAL KEY TO SUBORDERS OF MALLOPHAGA.

With filiform, 3- or 5-segmented, exposed antennæ; no labial palpi; mandibles vertical; œsophageal sclerite and accompanying glands usually present and normal; meso- and metathoracic segments fused; crop a saclike diverticulum; ingluvial glands present; testes four; egg tubes five..... ISCHNOCERA.

With clavate or capitate, 4-segmented, concealed antennæ; with 4-segmented labial palpi; mandibles horizontal; œsophageal sclerite and accompanying glands absent or modified; meso- and metathoracic segments with sutural line usually visible; crop simple; ingluvial glands absent; testes six; egg tubes three to five.

AMBLYCERA.

ANALYTICAL KEY TO GENERA OF THE SUBORDER ISCHNOCERA.

A With 3-segmented antennæ; tarsi with one claw; infesting mammals (family Trichodectidæ)..... *Trichodectes* Nitzsch.

AA With 5-segmented antennæ; tarsi with two claws; infesting birds (family Philopteridæ).

B Antennæ similar in both sexes.
C Meso- and metathoracic segments not fused..... *Nesiotinus* Kellogg.
CC Meso- and metathoracic segments fused.
D Front deeply angularly notched..... *Akidoproctus* Piaget.
DD Front convex, truncate, or rarely with a curving emargination, but never angularly notched.
E Species broad and short, with large, movable trabeculæ (at the anterior angle of antennal fossa).
F Forehead with a broad transverse membranous flap projecting beyond lateral margins of the head in the male, barely projecting in the female..... *Giebelia* Kellogg.
FF Without such membranous flap..... *Docophorus* Nitzsch.
EE Species elongate, narrow, with very small or no trabeculæ. *Nirmus* Nitzsch.
BB Antennæ differing in the two sexes.
C Species wide, with body elongate-oval to suborbicular.
D Temporal margins rounded; last segment of abdomen roundly emarginated; antennæ of male without appendage; third segment very long..... *Eurymetopus* Taschenberg.
DD Temporal margins usually angulated; last segment of abdomen convex, rarely angularly emarginated, with two points.
E First segment of antennæ of male large, sometimes with an appendage; third segment always with an appendage. *Goniodes* Nitzsch.
EE First segment of antenna of male enlarged, but always without appendage; third segment without appendage; last segment of abdomen always rounded behind. *Goniocotes* Nitzsch.
CC Species elongated narrow, sides subparallel.
D Third segment of antenna of male without an appendage. *Ornithobius* Denny.
DD Third segment of antenna of male with an appendage.
E Front deeply angularly notched.. *Bothriometopus* Taschenburg.
EE Front not angularly notched.
F Forehead with a broad transverse membranous flap or fold projecting beyond lateral margins of the head. *Philoceanus* Kellogg.
FF Without such membranous flap.
G Antennæ and legs long; a semicircular oral fossa. *Lipeurus* Nitzsch.
GG Antennæ and legs short; oral fossa narrow, elongate, extending as a furrow to the anterior margin of the head..... *Oncophorus* Rudow.

ANALYTICAL KEY TO GENERA OF THE SUBORDER AMBLYCERA.

A Tarsi with one claw; infesting mammals (family Gyropidæ)... *Gyropus* Nitzsch.
AA Tarsi with two claws; infesting birds (excepting *Boopia*?) (family Liotheidæ).
B Ocular emargination distinct, more or less deep.
C Forehead rounded, without lateral swelling; antennæ projecting beyond border of the head..... *Colpocephalum* Nitzsch.

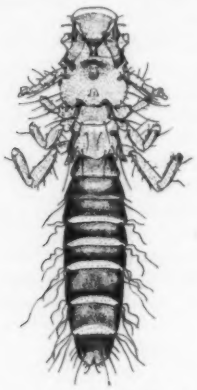
CC Forehead without strong lateral swellings.
D Antennæ projecting beyond border of the head; temporal angles projecting rectangularly; eye large and simple..... *Boopia* Piaget.
DD Antennæ concealed in groove on under side of the head; temporal angles rounded or slightly angular; eye divided by an emargination and fleck.
E Mesothorax separated from metathorax by a suture. *Trinoton* Nitzsch.
EE Meso- and metathorax fused; no suture. *Læmobothrium* Nitzsch.
BB Ocular emargination absent or very slight.
C Sides of the head straight or slightly concave, with two small projecting labral lobes..... *Physostomum* Nitzsch.
CC Sides of the head sinuous; forehead without labral lobes.
D Ocular emargination filled by a strong swelling; sternal markings forming a quadrilateral without median blotches.. *Nitzschia* Denny.
DD Ocular emargination without swelling, hardly apparent or entirely lacking; median blotches on sternum.
E Very large; with two 2-pointed appendages on ventral aspect of hind head; anterior coxæ with very long lobe-like appendages..... *Ancistrona* Westwood.
EE Small or medium; without bipartite appendages of hind head..... *Menopon* Nitzsch.

PHILOCEANUS gen. nov.

In a collection of Mallophaga taken by Mr. Rollo Beck from birds of the Galapagos Islands (the collecting of birds and parasites was done by Mr. Beck in the summer of 1901), are five specimens, including one male, three females, and one young, from a single specimen of *Procellaria tethys* (Wenman Id.) of a Mallophagan species not assignable to any of the known genera. The shape and habitus of whole body and the secondary structural differences between the sexes, shown in antennæ and abdominal segments, are those of *Lipeurus*, while the well-developed and unusual transversal membranous clypeal flap is that of *Giebelia*. The curious prolongation of the postero-lateral angles of the mesothorax is a character peculiar to the new genus. As *Giebelia* with its short, broad body and antennæ similar in both sexes stands to *Docophorus*, so the new genus, which may be called *Philoceanus*, with its elongate body, and differing antennæ, stands to *Lipeurus*.

The characteristics of the new genus may be given as follows: body Lipeuroid, elongate; head, thorax and abdomen of about

equal width (in widest places); antennæ differing in the sexes, that of male having an appendage on third segment; abdomen of male narrower than in female, parallel-sided, and with segments 6–8 each about twice as long as each of preceding segments; head with a broad, thin, transvenal, membranous clypeal flap projecting far on each side of forehead in an angulated and folded process; metathorax with postero-lateral angles conspicuously



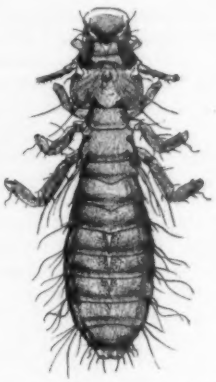


FIG. 1. *Philoceanus becki*, male. (Length, 1.6 mm.

FIG. 2.) *Philoceanus becki*, female. (Length, 1.5 mm.)

produced into tapering, blunt-pointed, backward-projecting processes.

PHILOCEANUS BECKI sp. nov. (Figs. 1 and 2.)

Five specimens (one male, three females, one immature) taken from *Procellaria tethys* (one specimen) Wenman Id. of the Galapagos group, summer of 1901, by Mr. Rollo Beck.

Description of Male.—Body, length 1.6 mm., width .27 mm. (abdomen), pale yellowish brown, with darker to blackish-brown marginal and transverse bands which cover so much of the surface as to give the posterior half of the body a general dark brown coloration.

Head, length .4 mm. width .3 mm., large in comparison with rest of body, wider than any other part of body, and conspicuously large, *i. e.*, wider and longer than the thorax; clypeal front broad, flatly convex and with distinct thin uncolored rounding margin; clypeal sutures distinct, broad, and with two conspicuous hairs at the marginal termination; these clypeal sutures form a V-shaped figure enclosing the distinct clypeal signature between the anterior prongs; the clypeus bears a conspicuous mem-

branous flap or fold, thin and uncolored, which rises from about in transverse line with the mandibles and projects forward to the point of the clypeal sutures, and laterally conspicuously beyond the margins of the head; in these lateral extensions the flap is folded back (towards the head margin) on itself; eye with rather long hair; the temples are not much swollen and each bears two long and a few short hairs; the antennæ (Lipeuroid) have the first segment as long as all the others combined and the third segment with an appendage; the ground color of the head is pale translucent yellowish-brown with the clypeal signature, a broad submarginal angulated band on each side of head, extending from clypeal suture to base of antennæ, darker brown.

Thorax small; prothorax with rounded postero-lateral angles with two separated longish hairs in each; metathorax a little wider and about twice as long, with postero-lateral angles conspicuously produced as thick, tapering, blunt pointed, finger-like processes, a long hair rising from base of each process and another not so long and two or three short ones rising from general postero-lateral angular region; posterior margin of metathorax slightly angulated in the middle and slightly concave in the space between this median angle and the postero-lateral angle; color pale translucent yellowish-brown with darker rather broad lateral margins.

Abdomen elongate, rather narrow, subparallel-sided; segments 1–5 each about one half as long as segments 6–8; long, flexible curling hairs in postero-lateral angles of segments 2–7, and terminal segment with many short fine hairs; pale yellowish-brown ground color almost wholly obscured by strong dark to blackish-brown lateral and transversal bands.

Female. — About same size as male but with abdomen wider (.4 mm.) in the middle and thus not parallel-sided; ground color of whole body less pale and translucent than in male; head with transversal clypeal flap as in male; antennæ without appendage on third segment and with first segment shorter than second; thorax with postero-lateral finger-like processes of meta-segment and with three or four long hairs in postero-lateral region; abdomen with second segment longest, others about equal among themselves, and segments 4–6 (in middle of abdomen) wider than others, so that the whole abdomen is elongate elliptical in outline; last segment with slight angular median emargination on posterior margin.

NESIOTINUS gen. nov.

A single female Mallophagan specimen of well-defined character received from Dr. G. Enderlein, of Berlin, proves to be a form which it is impossible to ascribe to any known genus of the order. This specimen was taken from *Aptenodytes longirostris*, a new penguin species from Kerguelen Id., collected by the German Deep-sea Expedition in 1899.

This new Mallophagan form unites in striking manner the important antennal characters of the family Philopteridæ with the general habitus and body characters of the family Liotheidæ. The shape of head, and the distinctly free metathoracic segment are characteristics heretofore peculiar to the genera *Menopon* and *Trinoton* (of the Liotheidæ), but the short, slender, five-segmented antennæ not lying in special antennal cavities identify the species as a Philopterid, but one not assignable to any known Philopterid genus. The new form represents a *Menopon*- and *Trinoton*-like genus in that family to which *Menopon* and *Trinoton* do not belong! The only other Mallophagan species taken from the penguin genus *Aptenodytes* is *Goniodes brevipes*, a small species very unlike this new form, described by Giebel (from a female specimen) in the *Phil. Trans. Roy. Soc.*, Vol. 168, extra Vol. This specimen also came from Kerguelen Id.

FIG. 3. *Nesiotes demersa*, female. (Length, 5 mm.)

The distinguishing characters of this genus are its *Menopon*-like form, the small suborbicular head with slightly-produced subrectangular temples, the distinctness of the meso- and meta-thoracic segments in a degree unequalled elsewhere among the known Mallophaga unless it be in *Trinoton*, the very small characteristically Philopterid antennæ, the sharp division of each eye into practically a pair of eyes, the large size of the hind body in comparison with the head, the heavy transverse blotches of the abdomen and the five pairs of abdominal spiracles instead of the usual six pairs.

NESIOTINUS DEMERSA sp. nov. (Fig. 3.)

Female. — Body, length 5 mm., width 2.1 mm.; head, length .75 mm., width 1.15 mm.; thorax, length 1.25 mm., width of prothorax .8 mm., width of mesothorax 1.30 mm., width of widest segment, the first, 2.16 mm.; chestnut brown, with large blackish-brown blotches on thorax and abdomen.

Head small in comparison with rest of body, hardly as wide as mesothorax, with flatly rounded front, no orbital sinus, temples slightly swollen, rounded, but with postero-lateral angle slightly obtusely produced, occipital margin slightly curving; eyes divided so as to give the effect of one pair on each side; antennæ short, slender, tapering; pustulated hairs on temporal margins and two small hairs with large pustulation on dorsal surface of each temple, also four smaller pustulations on postero-median dorsal surface, and one mesad from each eye pair; color chestnut-brown with blackish eye flecks and dark brown markings along temporal margin and in postero-mesial angles of each temporal region.

Thorax of three distinct segments regularly widening posteriorly, the meta-segment being nearly as wide as first (widest) abdominal segment and resembling an abdominal segment; prothorax with slight median angulated point on anterior margin, with parallel straight lateral margins and rounded antero-lateral and postero-lateral angles, anterior half dark brown, posterior half light brown; mesothorax with diverging lateral margins, small pustulated hairs in angles and flatly rounding posterior margin: anterior four fifths of segment dark brown with series of weak hairs in demi-pustulations along the hind margin of this dark region; metathorax with diverging lateral margins, and with large lateral transverse dark brown blotches leaving a rather narrow light brown median space. Legs with heavy short femora and long slender tibiæ with few short, weakly pustulated spiny hairs on each segment; two terminal tibial spines; femur darker than the translucent pale brown tibiæ.

Abdomen forming with meso- and metathorax an ellipse; segments 1 and 2 widest and others tapering slowly posteriorly; hairs few and inconspicuous; segments 1–5 with conspicuous spiracles each showing as a small brown spot in a large clear circular pustulation; segments 1–4 with large lateral transverse dark brown blotches leaving a lighter median space which is narrower on each successive segment posteriorly: segments 5–7 with dark-brown transverse bands extending clear across body; all transverse blotches and bands blacker and slightly wider at lateral ends, with slight anteriorly projecting process; indications of demi-pustulations in lateral portions of posterior margin of each blotch and band; posterior margin of terminal segment flatly rounded, and longest hairs of the body in lateral angles.

NOTE. — In a paper published while this paper was in press, on the Mallophaga from Birds of Costa Rica (Univ. Studies, Vol. 3, pp. 123–197, 1903, Univ. Nebraska) M. A. Carriker, Jr., describes two new genera of Mallophaga, under the names *Ornicholax* and *Kelloggia*.

EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF THE ORGANS IN THE EMBRYO OF THE FOWL (GALLUS DOMESTICUS).

FRANK R. LILLIE.

I. Introduction.

The results to be described under the above title relate to the morphology, functions and power of regeneration of various embryonic organs, and to the influence that certain embryonic parts exert on the development of others. They represent the application of a particular experimental method, viz., the destruction of definite parts, and study of the subsequent development. Thus the particular organs studied are those most accessible to operation, which form a rather heterogeneous assemblage. Nevertheless, taken as a whole, the results form a contribution to the subject of *correlative differentiation* of organs.

The Principle of Correlative Differentiation in Embryology (*i. e.*, influence of the intraorganic environment in development)[1] is that the rate, degree or mode of differentiation of any embryonic rudiment is dependent on some part or parts of the same organism (individual) external to itself; that is, that component parts of an embryo determine mutually to a greater or lesser extent, their respective lines and grades of differentiation. Much more is meant by this than that any embryonic part can develop only in its normal environment, which offers the prerequisites of its very existence. The principle of correlative differentiation in fact implies a distinction between *a determinative and a non-determinative environment*, and the problem of correlative differentiation is so far resolved when this is ascertained for all the organs (cf. Roux).

Any part, the entire environment of which is non-determinative, is said to develop by *self-differentiation* (Roux).

These two principles do not stand in the relation of rival theories but rather, probably, of coöperative factors in every

[1] Environment may be defined as conditions that influence dynamic processes in protoplasm, and may be divided into extraorganic and intraorganic, the former being external to the individual and the latter within its bounding surfaces.

embryonal differentiation, for any process of self-differentiation of a structure might be analyzable into correlative differentiation of its parts.

For the development of the higher animals at least the extra-organic environment is non-determinative. The development of the ovum as a whole is therefore a process of self-differentiation. But it is usually assumed that it is otherwise with the differentiation of its constituent parts; the extreme view being that each influences the mode of differentiation of all the remainder. From this standpoint the complexity of the correlative processes of differentiation must increase in proportion to the increase in complexity of structure.

Theoretically, at least, the determinative value of correlative differentiation in any case may be (1) absolute, *i. e.*, the mode of development of a part being determined entirely from without itself; (2) partial; (3) wanting, *i. e.*, absolute self-differentiation.

Our present knowledge is enough to exclude the first theoretical possibility. No principle in embryology is better established than that sooner or later the embryo is a mosaic of embryonic rudiments, each of which is to a certain extent self-determining. This mosaic of rudiments may become visible very early, as in those ova exhibiting a definite cell-lineage of organs, or it may appear later. In some cases, at least, the unsegmented ovum itself is a simple mosaic (ovum of ctenophores according to Fischel; ovum of *Unio*, Lillie; ovum of sea-urchins, Boveri; ovum of frog, Roux, Schulze and others). Indeed it is quite probable that all ova are more or less simple mosaics of embryonic rudiments.

Unless, therefore, we wish to beg the entire question we must proceed on the second hypothesis. This is the writer's standpoint, and the problem is to determine as many definite correlations as possible and to investigate their nature.

There is probably no conception in embryology so vague as that of correlative differentiation, as the following citations may serve to show:

Hertwig: "Zelle und Gewebe," II.:

"Die Wechselwirkungen (Correlationen) zwischen den Zellen eines Organismus und ihren Derivaten bilden sich mit dem

Beginn des Entwicklungsprocesses aus, ändern sich von Stufe zu Stufe und compliciren sich in demselben Maasse, als die Entwicklung fortschreitet.

" Im Gegensatz zum Mosaiktheorie von Roux und der keimplasma theorie von Weismann stellt die Theorie der Biogenesis den Grundsatz auf, dass vom ersten Beginn der Entwicklung an die durch Theilung des Eies sich bildenden Zellen beständig in engster Beziehung zu einander stehen, und dass dadurch die Gestaltung des Entwicklungsprocess sehr wesentich mit bestimmt wird. *Die Zellen determiniren sich zu ihrer späteren Eigenart nicht selbst, sondern werden nach Gesetzen die sich aus dem Zusammenwirkung aller Zellen auf den jeweiligen Entwicklungsstufen des Gesammtorganismus ergeben, determinirt.*"

Herbst: " Formative Reize in der Tierischen Ontogenese: "

" Die Aufgabe des zweiten Teiles meiner Abhandlung über die formativen Reize war es also, in der tierischen Ontogenese, abgesehen von der Namhaftmachung jener wenigen Fälle von Gekommen von formativen Reizwirkungen, die von irgend einem Teil des Organismus auf einen oder mehrere andere ausgeübt werden, festzustellen und eventuell die Möglichkeit der vollständigen Auflösung der ganzen Ontogenese in einer Reihe von solchen Induktionserscheinungen nachzuweisen.

" So ist es zum Beispiel zum mindesten ungenau, von der 'weitgehenden Wechselbeziehung' zo sprechen, 'die zwischen allen Teilen eines Organismus auf allen Stadien seiner Entwicklung besteht' (Hertwig: 'Evolution und Epigenesis'); denn das Ektoderm der Echiniden entwickelt sich unabhängig vom Entoderm, und auch abgeschnürte Hautstücke, etc., konnen sich selbständig differenzieren, wie dies das Vorkommen der Teratome beweist (Roux). Die Annahme einer ganz allgemeinen Korrelation zwischen allen Teilen des Organismus auf allen Stadien der Ontogenese ist deshalb ebenso falsch wie jene von der qualitativ ungleichen Kernteilung der Mosaiktheorie."

Most of the real illustrations (*i. e.*, experimentally determined) of this principle must be taken from plants and plant-like animal colonies. One need only glance through Herbst's recent " Formative Reize in der Tierischen Ontogenese " to realize that, so far as egg development is concerned, the application of the prin-

ciple rests very largely on inference, analogy and a few doubtful pathological conditions.

Discussion of this subject belongs, however, to the conclusion rather than to the introduction, and the foregoing remarks are intended only to define the problem.

II. Methods of Operation.

In making the operations one must work as far as possible under antiseptic conditions. Instruments, etc., must be sterilized; this is most readily done by passing the needles, knives, scissors, etc., through a flame immediately before each is used. In spite of all precaution a great many eggs are infected. In my experiments only about 20 per cent. of the eggs remained alive until the time of examination for the results of the experiment, two to five days after the operation. The causes of the mortality in the remaining 80 per cent. are two: (1) Fatal injury of the operation (about 40 per cent.?); (2) infection with mould or bacteria (about 40 per cent.?). There is a very noticeable difference between different lots of eggs; some bear operations much more readily than others and are less prone to infection. These differences in the relative powers of resistance of different lots of eggs are due to the relative freshness of the eggs when incubation is begun, and also to the time of year. It is noticeable that in a lot of eggs in which a relatively large proportion, over 50 per cent., fail to develop in the incubator, the percentage of failures in the actual experiments is usually very high.

The method of procedure in my experiments was as follows:

1. The eggs are not turned in the incubator, so that one may be sure of locating the position of the embryo in the unopened egg exactly. The upper side of each egg is marked with a pencil.

2. A small opening is made through the shell and membrane over the embryo.

3. The operation is then made. For cauterization I employ either a needle heated red hot in the flame, or an electric cauterizing needle. The heated needle cools very rapidly, so that the operation must be hastily performed, and it is difficult precisely to delimit the injury. The electric cautery, on the other hand, is apt to give too intense heat. Each method possesses certain advantages.

4. The opening in the egg is closed as follows: A piece of the shell with membrane attached is cut from a corresponding part of another fresh egg, so as to be slightly larger than the opening in the operated egg. This is placed over the opening so as to close it completely; and the albumen adhering to the membrane acts as cement. To ensure perfect closure strips of the egg-membrane are plastered on so as to overlap all edges of the foreign shell. The advantages of this method of closure are that the foreign surfaces are perfectly aseptic if fresh eggs are used, and that the conditions are as nearly like the normal as possible. It is, morover, the simplest and easiest method. This method of closing the opening was first used by Miss Peebles.[1]

III. Experiments on the Amnion and the Production of Anamniote Embryos in the Chick.

A. *The Normal Development of the Amnion.*

The purpose of this section is to give a brief statement of some facts concerning the formation of the amnion before taking up the analysis of the processes by experiment. This is necessary because the facts are at least partly new, and without knowledge of them the mechanics of formation of the amnion cannot be understood. For a recent review of the literature on the whole subject of the amnion in the Sauropsida, see Schauinsland ('02*a* and '02*b*); the latter paper I regret not to have seen.

In the somatopleure on each side of the axis of an early embryo of the chick three zones may be distinguished on the basis of the subsequent differentiation, (*A*) for the body-wall; (*B*) for the amnion; (*C*) for the chorion (serosa) (Fig. 1). It is important to trace the origin of the differentiation between the amnion and serosa on the one hand, and amnion and body-wall on the other, for the conditions that determine the development of the amnion must be antecedent to such differentiation.

1. *The Ectamnion.*—The differentiation of the amniogenous from the choriogenous somatopleure is always preceded by the appearance of a thickening of the ectoderm along the external margin of the former. This thickening, for which I propose the

[1] *Roux's Archiv*, VII., 1898.

name ectamnion, precedes by a little the formation of amniogenous folds in any region, and indeed it induces the origin of the entire system of folds. It has been described by many embryologists at the stages immediately preceding fusion of the limbs of

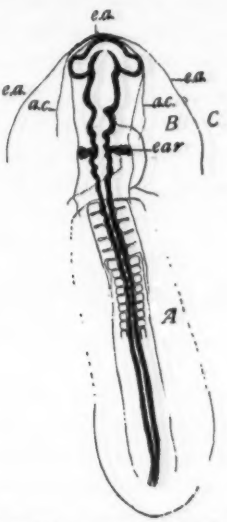


FIG. 1. Embryo of chick with 13 mesoblastic somites. University of Chicago Embryological Collection, No. 555. *e.a.*, ectamnion; *a.c.*, inner margin of amniocardiac vesicles; *A*, region of the somatopleure destined to form the body-wall; *B*, amniogenous somatopleure; *C*, choriogenous somatopleure.

the amnion (cf. Schenk, '71), and it forms the ectodermal seroamniotic connection of Hirota ('94). But no one, so far as I know, has traced it back to its origin and recognized the fact that it is the earliest formed part of the amnion, which is thus primarily ectodermal in the chick, as in Chelonia and some other primitive Sauropsida.

The ectamnion may first be distinguished at about the stage with nine mesoblastic somities, where it appears as a median thickening of the ectoderm in front of the head near the anterior boundary of the proamnion. Along the line of this thickening there is a fusion, between ectoderm and entoderm. The thickening is extended right and left and turns backwards along opposite sides of the head to about the region of the middle of

the heart, gradually becoming more peripheral in position and slowly fading out (Fig. 1). This line represents the junction of the amniogenous and choriogenous somatopleure, and thus corresponds to the angles of the future amniotic folds.

The head of the embryo lies in a depression bounded in front by the ectamnion and on the sides by the amnio-cardiac vesicles of the body cavity, along the inner upper margin of which the ectamnion runs for a short distance. The floor of the depression is the proamnion.

In a stage with 14–15 mesoblastic somites the ectoderm of the proamnion is much more thickened in front of the head, and has

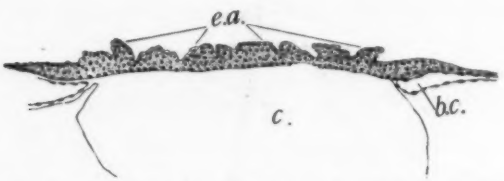


FIG. 2. Transverse section through the anterior angle of the ectamnion, a few sections in front of the tip of the head. 14–15 mesoblastic somites. University of Chicago Embryological Collection, No. 215. *b.c.*, body-cavity; *c.*, large cavity in the entoderm; *e.a.*, ectamnion.

a *villous outer surface* in consequence of irregularity in the thickening[1] (Fig. 2), which may be traced back to the level of the heart, and on one side to its hinder end; there is also a very short ectentodermal fusion beneath the tip of the head. In this series the ectamnion marks the boundary between two distinctly differentiated parts of the extraembryonic somatopleure, the more central of which is the amnion.

In another embryo with fourteen mesoblastic somites, the tip of the head is surrounded by the amnion, and the proamniotic part is represented only by a short median strip extending eight sections back to a point where the limbs of the amnion have not yet closed. The ectamnion is continued only for a short distance along the *angles* of the amniotic fold, and then passes peripher-

[1] In examining the section one receives a strong impression that the irregularities may be due to amœboid movements; but it is not possible to confirm this by actual observations.

ally. How has the head-fold been formed? The great expansion of the body cavity (amniocardiac vesicles) on each side causes an elevation of the anterior angle of the ectamnion and a pocket is formed by fusion of its opposite limbs, which have a strong affinity for each other; fusion proceeds along the median dorsal line so long as the energy of fusion is sufficient to draw the somatopleure up. The head of the embryo is rapidly elongating at this time and slips into the pocket thus formed, being guided in part by the cranial flexure (Fig. 1). It is interesting to note how far the ectodermal thickening stretches ahead of the mesoderm of the fold near the point of closure, and that the apical cells are elongated into pseudopodium-like processes.

The histological differentiation of the amniotic area of the somatopleure from the chorionic portion precedes the elevation of the fold.

This brief inquiry, then, suggests that the order of events in the formation of the head fold of the amnion is:

1. Thickening of the ectoderm on the outer margin of the amniogenous somatopleure, beginning in front of the head of the embryo and extending back on each side (ectamnion).

2. Great expansion of the body cavity on each side opposite the head of the embryo and consequent elevation of the anterior bay of the ectamnion to the level of the dorsal surface of the embryo.

3. Fusion of the right and left limbs of the ectamnion, beginning at the angle, to form a pocket, the head-fold of the amnion.

4. Pushing of the head of the embryo into the fold.

There may be, however, considerable variation in the time of formation of the head-fold. I have, for instance, one series with 17–18 mesoblastic somites (ser. 175), where the head-fold is not yet formed.

Extension of the Ectamnion. — The ectamnion differentiates backward more rapidly than the lateral folds, and always precedes their origin. In the 48-hour stage (21–22 somites) (Fig. 3) the ectamnion from in front has joined that from behind formed in connection with the tail-fold. There is a place, corresponding nearly to the final meeting place of anterior and posterior lateral folds, where it becomes very faint. It would appear

then that behind the tail there is actually a new starting-point for the ectamnion as well as the amniotic folds. The primary position of the ectamnion is near the boundary of the pellucid area; towards the posterior end it bends in very sharply, nearly joining the body wall proper, and terminating in the posterior rudiment.

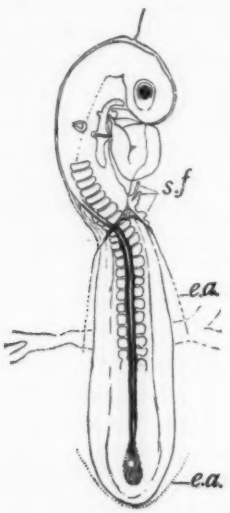


FIG. 3. Embryo of chick with 21 mesoblastic somites. University of Chicago Embryological Collection, No. 99. *e.a.*, ectamnion; *s.f.*, secondary folds of the amnion on the right side. The dotted line continuing *e.a.* represents the continuation of the ectamnion beyond the region of folding. The dotted area at the angle of the folds represents the ectodermal sero-amniotic connection of Hirota.

Origin of the Tail-Fold. — The tail-fold proper arises from an ectodermal thickening lying in a depression just beneath the rudimentary tail-bud. The depression is caused by the enlargement of the body cavity on each side of the middle line. These enlargements may be called the amnio-allantoic enlargements, as they are associated with the formation of the allantois. I would venture the hypothesis that the existence of a *separate* tail-fold of the amnion is associated with the time of development of the allantois, which is represented in the embryo under consideration (1) by a shallow entodermal evagination and (2) a mass of mesoblast.

At the time of formation of the tail-bud a very shallow pocket forms behind it. This owes its origin to the elevation of lateral folds of the somatopleure and progressive fusion beginning at the posterior angle of the ectamnion. The floor of the pocket includes a thick posterior prolongation of the allantoic mesoblast which furnishes a firm floor to the pocket and thus determines the form of the folds.

2. *The Amniotic Folds.* — The subsequent development includes the elevation and fusion of the anterior and posterior lateral folds. The final closure takes place opposite the buds of the hind limbs. The order of events in these processes is as follows:

1. The growth of the amniogenous somatopleure behind the head-fold and in front of the tail-fold.

2. The uprising of the amniotic folds, and their growth in a definite direction around the embryo.

3. The fusion of the right and left folds along the line of the ectamnion in such a way that the external limbs unite to form the chorion, and the internal to form the amnion.

Study of the morphology of these processes suggests the following physiological conclusions:

1. The growth of the amniogenous somatopleure may be a result of the traction exerted in it by the progressive fusion of the folds already formed in front and behind.

2. The uprising of the lateral folds is determined by the head- and tail-folds, the progressive fusion of the right and left ectamnion dragging the amniogenous somatopleure into place.

It remains to test these conclusions by experiments, but before proceeding to a description of these, I wish to describe *the influence of the rotation of the embryo on the amniogenous somatopleure.*

Practically all of the somatopleure of the pellucid area is amniogenous with the exception, naturally, of that part internal to the limiting sulci that forms the body-wall. What effect has the turning of the embryo on its left side on the amniogenous somatopleure? We will suppose that the latter is primitively of equal width on both sides; we will furthermore assume that the somatopleure cannot be drawn in from the vascular area, because it is here attached to the splanchnopleure. (The fusion of the somatopleure and splanchnopleure at the margin of the pellucid

area is shown by the fact that the splanchnopleure is often drawn up with the outer limb of the amniotic fold, making a fold of the splanchnopleure at this place) (Fig. 5). Finally let us assume that the notochord represents approximately the axis of rotation. During the process of rotation the embryo sinks and the lateral limiting sulci become deeper. A direct consequence of the rotation must be therefore a strong tension on the somatopleure belonging to the under (left) side, *a-b*, and practically none on the upper (right) side, *c-d*, (see Fig. 4, *A*, *B*, *C*).

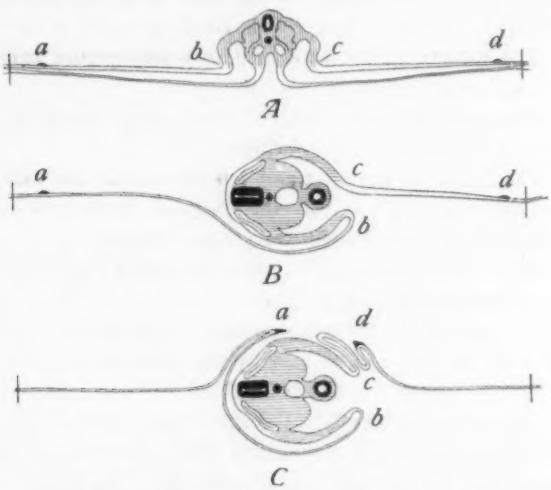


FIG. 4. *A*, *B* and *C*. Diagrams to represent the effect of rotation of the embryo on the amniogenous somatopleure. *a* represents in all figures the position of the ectamnion on the left (lower) side; *d* represents in all figures the position of the ectamnion on the right (upper) side. *b* and *c* represent the junction of amnion and body-wall on left and right sides respectively. In Fig. *A*, *a–b* and *c–d* are equal. In Fig. *B*, rotation of the embryo is assumed to have taken place without formation of the amnion; the distance *a–b* has become greater than *c–d*. In Fig. *C* is represented rotation of the embryo with synchronous formation of the amniotic folds, as is actually the case; *c–d* is inevitably thrown into secondary folds. The vertical lines at the extreme right and left represent the margins of the pellucid area.

Even though the difference may be partly compensated for by drawing of the embryo to the left, the tendency would be to stretch *a-b*. If there were no such compensation and *a* and *b* were practically fixed points, the length of *a-b* at the conclusion

of the rotation would much exceed that of *c-d* (Fig. 4, *b*); and if during this process there were actual independent growth of *a-b* and *c-d*, the latter would of necessity be thrown into folds, but not the former. Finally, if the amniotic folds were forming at the same time (as is actually the case) the right one would inevitably be thrown into secondary folds by the approximation of points *c* and *d* (Fig. 4, *C*).

Study of the fusion of the amniotic folds in actual section shows (1) that the line of fusion of the opposite amniotic limbs is over the dorsal surface of the embryo *only so long as the latter lies flat on the yolk*, and does not follow the turning of the embryo on to (usually) its left side; the consequence is that after rotation of the embryo the line of fusion lies over the upper (right) side of the embryo, often opposite the horizontal level of the intestine

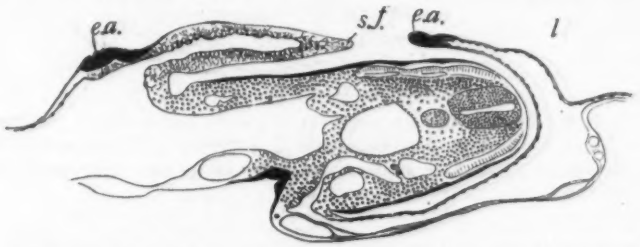


FIG. 5. Transverse section of an embryo of about 48 hours (Duval) showing the position of the ectamnion on the right and left sides. University of Chicago Embryological Collection, No. 689. *e.a.*, ectamnion; *l.*, left; *s.f*, secondary fold of amnion on the right side. The great differences in the thickness of the amnion of the right and left sides should be noted.

(Fig. 6). Thus one fold of the amnion passes all the way from the under side over the back of the embryo and around on the other side to the line of fusion, and thus is several times as long as the opposite limb. (2) Moreover, the amniotic fold of the right side is invariably thicker than that of the left side, and is always thrown into secondary folds at the place of turning (Fig. 5 and Fig. 6). These conditions are satisfactorily explained, as noted above, by the mere turning of the embryo on its side.

One must therefore distinguish in the upper limb of the amnion two kinds of folds: (1) The ordinary amniotic fold induced by the fusion of the right and left rudiments and (2) *secondary*

folds formed simply by the process of twisting of the embryo. This distinction is of importance in interpreting the results of the experiments.

Hirota (94) notices the secondary fold on the upper side and says: "It seems to owe its origin to the presence of the sero-amniotic connection. . . . It is always on the right side of the connection, and is pushed on towards the left. There takes place no folding before the allantois appears, and the longitudinal ex-

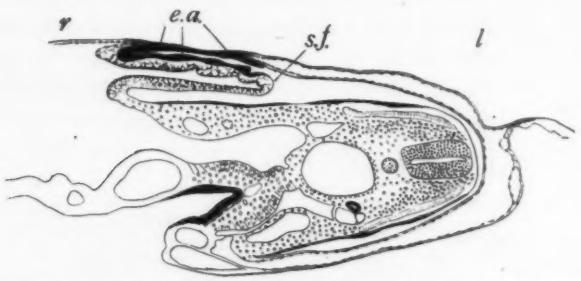


FIG. 6. Section of the same embryo as the preceding, 10 sections (150μ) in front of Fig. 5. The section passes through the place of fusion of the right and left folds. The secondary fold of the amnion is well shown on the right side. Letters as in Fig. 5.

tent of the fold depends on the extent of the sero-amniotic connection." "Its form and extent are variable." "It is not clear what significance this fold has." "At both extremities of the sero-amniotic connection the amnion is also slightly folded longitudinally."

These secondary folds of the amnion are very transitory except in two regions: (1) Above the hind end of the heart (apex of ventricle) and continuing a short distance behind it; (2) in the region immediately in front of the allantois, at 60–70 hours, thus in the neighborhood of the final closure of the amniotic folds. The former are of very constant occurrence and persist a long time (Fig. 3). The latter are relatively slight and inconstant. Hirota is thus mistaken in saying that these folds do not appear until the formation of the allantois.

The secondary folds in the neighborhood of the heart are always on the upper (right) side; they first appear at the time

of rotation of the embryo, and are coincident with the closure of the amnion (Fig. 3); they persist until the body-wall is completed behind the entire heart. They are not, in my opinion, exclusively folds of the amnion, but extensions of the body-wall for enclosure of the region of the heart and liver. The direct cause of their formation is, however, the rotation of the embryo with extreme growth of the body-wall contiguous to the amnion, and fixation of the outer end of this limb of the amnion by the amniotic suture.

Elsewhere the effect of the twisting of the embryo is rapidly compensated so that the secondary folds of the right half of the amnion do not persist long except in the region of the allantois, where slight inconstant secondary folds may continue longer.

B. *Experimental.*

1. *Experiments on the Head-fold of the Amnion.*

Experiment No. 57.

Age of the embryo at the time of operation, 33 hours[1] (Duval).

Operation.—The blastoderm was cauterized lateral to the right optic vesicle with a needle (Fig. 7) so as to make a large opening. At the time of the operation only the most anterior horse-shoe-shaped segment of the ectamnion was present (cf. Fig. 1), and this was destroyed only on the right side of the embryo. On the left side, therefore, the amniotic fold was free to form to the extent that it is independent of the opposite fold. The right optic vesicle was slightly injured, as the results of the experiments show. In opening the egg for the operation, the blastoderm was

[1] In describing the various experiments, the age of the embryo at the time of the operation will not be given as the actual number of hours in the incubator, because the variations in point of actual development after the same period of incubation are so extreme. It is not possible either to make accurate measurements of the living embryo or to determine the number of somites present, on account of the loss of time and danger of exposure of the embryo. A rough sketch of the embryo was always made at the time of the operation, and this is sufficient to identify it with the various stages figured in Duval's atlas. The age is based on this identification. Thus the given age at the time of operation in these experiments represents a certain definite stage of development. On the other hand, the length of time that elapsed from the experiment to the time of reopening the egg is always given literally.

also inadvertently torn just back of the embryo, and this opening also appears in Figs. 8 and 9. This, however, was without any noticeable effect on the subsequent development.

FIG. 7. Experiment 57. Operation diagram. Outline of embryo of chick of about 33 hours, after Duval. The ruled area to the right of the head indicates the area of the blastoderm destroyed by the heated needle.

Examination of the Resulting Embryo.—The egg was reopened 48 hours after the operation. The heart was beating vigorously; the hole made in the blastoderm by the operation had not closed, and a good deal of yolk had escaped through this and overlay the blastoderm. The embryo was well developed, corresponding to the stage of 70–80 hours (Duval), and apparently normal in all essential respects. (A defect in the right eye was evidently a direct result of the operation.) The head of the embryo had slipped through the hole in the blastoderm and was suspended in the yolk (Figs. 8 and 9).

The embryo was cut into 250 sections of 15 μ thickness. Around the edges of the opening made by the operation the somatopleure turns over and becomes continuous with the splanchnopleure, ectoderm with entoderm, and mesoderm with

mesoderm. In places one cannot determine where the ectoderm leaves off and the entoderm begins.

Amniotic Rudiments of the Left Side. — A short distance in front of the margin of the opening there is a sharply defined fold of the somatopleure capped by an ectodermal thickening that

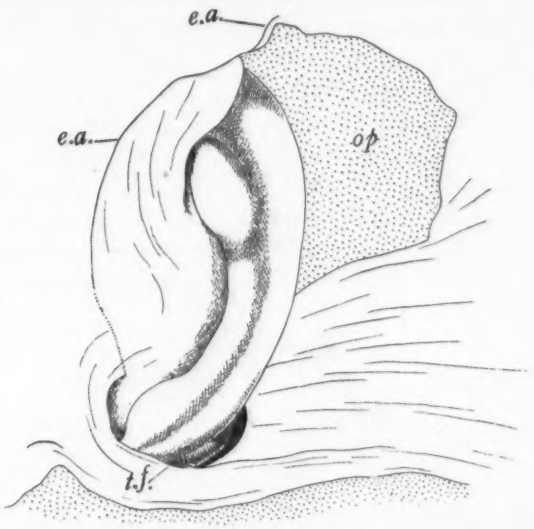


FIG. 8. Experiment 57. Upper surface of blastoderm. *op.*, aperture in the blastoderm made by the operation; *e.a.*, amniotic rudiment of the left side; *t.f.*, tail-fold of the amnion. The stippled area behind the embryo represents an aperture in the blastoderm accidentally made in opening the egg for the operation.

represents the head-fold and left lateral fold of the amnion. The extent of this fold is indicated by the line *e.a.* on Fig. 8. It begins as a sharply marked fold at the most anterior angle of the opening, and passes back, at first along the edge of the opening, later a short distance from it, to the left of the embryo. It very distinct (Fig. 10, *l.a.f.*) to the point where it is indicated as is a broken line; in this region the fold has disappeared, but the thickening of the ectoderm (ectamnion), may be traced back to the tail-fold with which it becomes continuous as indicated in the drawing (Fig. 8). At no place, until the tail-fold is reached, is the somatopleure internal to this line thrown into folds. By

reference to the figure and to the description of the operation it will be seen that the line of this fold represents the continuation of the left amniotic rudiment, which was not injured by the operation.

I conclude, therefore, that when the amniotic rudiment of one side is left free to develop after destruction of the rudiment of the other side just prior to the formation of the head fold,

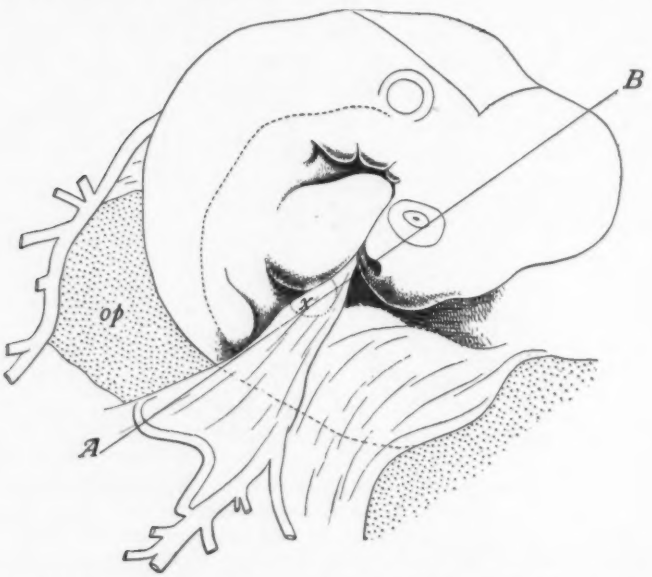


FIG. 9. Experiment 57. Under surface of the blastoderm. There is no amnion. The right eye is defective. *x* marks the location of the secondary amniotic fold shown in Fig. 10. *A–B*, plane of section shown in Fig. 10. Letters as in Fig. 8.

the ectamnion is propagated in the normal fashion and induces the formation of a low fold, but that the amniogenous somatopleure is unable to raise itself around the body of the embryo. The growth of the amniogenous somatopleure appears to be less than normal.

Amniotic Rudiments of the Right Side. — On the right side, on the other hand, a well-developed fold appears at the place where the extra-embryonic somatopleure becomes continuous with the

body-wall (section 138, Fig. 10) and extends to section 167, where it suddenly ceases, a distance of about 0.5 mm. The location is indicated by *x* on Fig. 9, and Fig. 10 shows it in section.

The formation of this fold is not induced by the ectamnion because the line of the latter (Fig. 10, *r.e.a.*) may be recognized some distance lateral to the fold, through it is very slightly developed. The fold in question is immediately back of the heart on the right side of the body. It is not, in my opinion, a true amniotic fold, but belongs to the category of normal secondary folds of the amniogenous somatopleure produced by the turning of the embryo, with which it agrees precisely in position and appearance. This conclusion is reinforced by the following consideration : in this embryo the roots of the vitelline veins are prolonged forward to an abnormal extent, and the right vein is fused to the somatopleure lateral to the fold (Fig. 10). As the

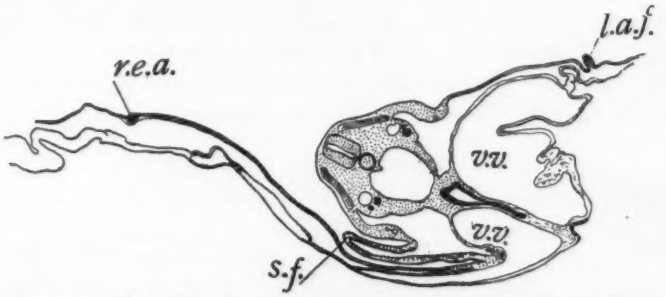


FIG. 10. Section through the embryo of experiment 57 along the line *A–B* of Fig. 9. *l.a.f.*, left amniotic rudiment; *r.e.a.*, ectamnion of the right side; *s.f.*, secondary fold of amnion on the right side; *v.v.*, vitelline veins.

embryo turns, therefore, the somatopleure between the vitelline vein and the body-wall must be folded to the extent that the turning approximates the body-wall to the vein, because the fusion prevents the somatopleure from being pushed peripherally. As already said, therefore, this is not a true amniotic fold.

The prevention of the formation of the head-fold, by destruction of the rudiment of one side, operates to prevent the normal elevation of the amniotic fold on the opposite side; and thus it is experimentally demonstrated that the coöperation of right and left folds is necessary for the normal mode and direction of

growth of the amniotic rudiments. The height of the fold on the uninjured side is a measure of the power of independent elevation of a single amniotic fold.

On the other hand the existence of the ectamnion on the right side, though in a rudimentary state, and the differences in finer structure of the somatopleure on the two sides of this line indicate that the distinction between amniogenous and choriogenous somatopleure is attained by the normal development of the somatopleure as a whole, and not simply as a result of their separation after fusion. However, the relatively rudimentary condition of the ectamnion on the injured side shows that the earlier stimulate the growth of the latter formed parts; otherwise we should expect to find the ectamnion equally developed on both sides. The ectamnion of the right side does not exactly join the tail-fold.

Tail-fold. — The tail-fold of the amnion may be well seen in Fig. 8. So far from compensating in any way for the absence of head and lateral folds, it is of even less than its normal extent, a fact indicating (possibly) that normally its growth is stimulated by the traction of the anterior section of the amnion.

Experiment No. 36.

Age of the embryo at the time of operation forty-six hours (Duval).

Operation. — The operation consisted in the insertion of a heated needle just in front of the heart (see Fig. 11). Examination of the sections of the resulting embryo shows that the injury involved the left optic cup slightly, and that the head-fold of the amnion which extends back beyond the heart at this stage, stuck to the needle and was stripped off, carrying with it a certain amount of the adjacent somatopleure. This was not observed at the time of the operation, but the conclusion is rendered positive by the subsequent examination of the embryo.

Examination of the Resulting Embryo.

The egg was reopened and the embryo preserved forty-eight hours after the operation. The embryo (Fig. 12) appeared like a normal embryo of about the ninety-sixth hour. The limb-buds were well started, and the allantois extended out beyond

the embryo, but towards the dorsal surface; the flexures were normal. The striking thing was the apparent entire absence of the amnion; the embryo lay naked on the surface of the blastoderm, to which it was attached, in the same manner as a selachian embryo by a very broad somatic and splanchnic umbilicus.

In the normal embryo of this age the amnion is completely closed, and the body-wall of the embryo has, therefore, lost all connection with the chorion.

FIG. 11. Experiment 36. Operation diagram. Outline of embryo of chick of about 46 hours, after Duval. The ruled area shows the site of the operation with the heated needle. For description of the operation see text.

This embryo was cut into 625 transverse sections. These confirm the general absence of the amnion, and at the same time furnish additional data. Back to about the 354th section (forty sections behind the heart), the somatopleure beneath the embryo is entirely missing; evidently it had been torn away by the operation and had not been replaced. Throughout this region the extra-embryonic somatopleure begins on each side of the embryo with a free edge. A short distance behind the heart, folded portions of the original amnion appear lying in the gap in the somatopleure, and continuous with the midventral line of the body-wall. Beginning with about the 371st section (see Fig. 13) the body wall is open ventrally, and is continuous with the

extra-embryonic somatopleure on one side, while on the other the original gap in the somatopleure is still open (see Fig. 13). In this region, the somatopleure for some distance external to

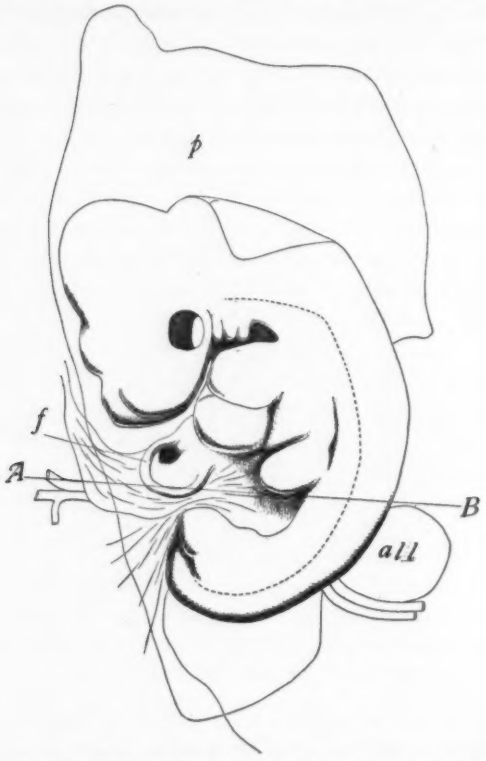


FIG. 12. Experiment 36. Surface view of embryo; upper surface of blastoderm. The embryo is anamniote, except for a rudimentary tail-fold. *all.*, allantois; *p.*, pellucid area. *A–B*, plane of section shown in Fig. 13; *f.*, fold of somatopleure.

the part destined for the body-wall is thrown on both sides into irregular folds that obviously represent the lateral amniotic folds. They rapidly decrease in size posteriorly, and *almost* completely disappear in the region extending from the 420th section back, *i. e.*, a short distance back of the fore-limbs. Beginning opposite the hind-limbs the folds again increase in size. They are very irregular and do not form the normal investment

of the tail. But beneath the latter they form a closed pocket, the usual tail-fold.

Over the entire region, extending from about the posterior edge of the fore-limb to the beginning of the hind-limb, there are no folds in the amniogenous somatopleure. This would indicate that the normal rapid growth of this region is progressively induced under normal conditions by the extension of the lateral angles of the head-fold backwards. The folds shown in the figure are only from about 354–430 and may be explained as remnants of the original head-fold, the postero-lateral prolongations of which were probably not entirely removed by the operation. These folds have not, however, united over the embryo nor have they induced formation of folds behind them. The

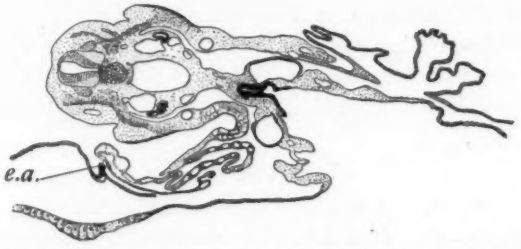


FIG. 13. Experiment 36. Section of embryo along the line *A–B*, Fig. 12. The irregular and incomplete amniotic folds are well shown. *e.a.*, ectamnion of the right side.

reason for this is clear when we consider that the normal process involves continuous traction on the somatopleure back of the advancing folds, for the latter are continually fusing along the dorsal line with those of the opposite side and thus are constantly, so to speak, gathering in the slack, and causing tension.

In the drawing of the entire embryo, the left side is uppermost, but at the time of the operation the right side was up. Evidently the embryo was turned over after removal of the blastoderm in the process of preparation. This explains why in the section the lower amniotic fold has the usual appearance of the upper fold. The ectamnion is visible only on the left side of the drawing; on the right side no trace of it could be found, except in the region of the tail-fold.

The results of the destruction of the head-fold of the amnion in the stage of 46 hours are: (1) Inhibition of the progressive differentiation of the amniotic zone of the somatopleure; (2) failure of the parts of the lateral folds left to unite around the embryo. The failure of the amniotic folds to unite in the region where they are best formed and are of more than sufficient length for enclosure of the embryo shows that the normal union of the folds is due to the guidance and support of the earlier formed parts of the amnion.

The tail-fold, however, forms in a fairly normal manner. The actual abnormalities in this fold are probably secondary, that is, probably due not so much to direct disturbance of the amnion itself as to the freedom of movement of the embryo permitted by the absence of the head-fold, resulting in the withdrawal of the tail of the embryo from the forming tail-fold.

The body-wall is unenclosed for 113 sections; in a normal embryo of about the same age the body-wall is unenclosed for about 55 sections. Thus it would appear that the closure has been delayed.

Experiment 60.

Age of the embryo at the time of operation about 33 hours.

Operation.—The blastoderm was cauterized just lateral to the right optic vesicle, as in experiment 57, producing a large opening (Fig. 14).

The egg was reopened 72 hours after the operation, and a large, finely developed vascular area was seen with apparently no embryo. But more careful examination revealed the naked hind quarters of an embryo sticking up near the center of the vascular area, the whole trunk and head of which were plunged through the blastoderm into the yolk. The head and trunk of the embryo had slipped through the hole made by the operation into the yolk-sac, and the edges of the blastoderm around the original opening had fused in such a way as to close around the hinder part of the embryo. A large part of the vascular area was cut out and the embryo was gently floated into a watch crystal of physiological salt solution. Turning over the blastoderm, the embryo was revealed entirely without an amnion (Fig. 15). Not even the tail-fold was found.

The embryo is represented in Fig. 15 as it lies on the reversed blastoderm, the entodermal face of which is up. The allantois is well developed and lies in a special enlargement of the body cavity behind the embryo.

FIG. 14. Experiment 60, operation diagram. Outline of embryo of chick of about 33 hours, after Duval. The ruled area indicates the region of the blastoderm destroyed by the operation.

In this experiment, as in experiment 57, only the right limb of the ectamnion of the prospective head-fold was destroyed; and the consequence of this is in both cases the suppression of the amnion with the exception of the tail-fold. In this case the various membranes have been so confused by the curious position of the embryo and by various secondary fusions that it is quite impossible to determine the behavior of the uninjured rudiment of the amnion of the left side. A single section may serve to illustrate one of the very peculiar conditions (Fig. 16). Lying above the embryo is seen the blastoderm composed of the somatopleure and splanchnopleure. The body-wall of the embryo has fused with the splanchnopleure in such a way that the two are directly continuous on both sides, and the body wall may be traced directly into the wall of the intestine. The result

of this fusion must have been an opening on each side into the yolk-sac; but this has been roofed over by extension of the

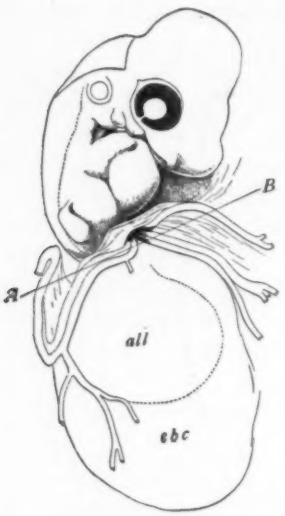


FIG. 15. Experiment 60. Under *i.e.* entodermal, surface of the blastoderm. The embryo is anamniote, but otherwise quite perfect. *all*, allantois; *e.b.c.*, extra-embryonic body-cavity; vitelline arteries and veins shown. *A-B*, plane of the section shown in Fig. 16. The embryo was suspended within the yolk-sac, as described in the text.

blastoderm surrounding it. Farther back the wall of the intestine becomes continuous with the extra-embryonic splanchnopleure.

In the region of the tail rudiments of the tail-fold of the amnion are found.

Two other completely anamniotic embryos (numbers 112 and 124) were produced by experiments similar to those already described. Both of these had passed through the hole made in the blastoderm and were suspended within the yolk-sac. One of these was much farther developed than number 60. They confirm the general results of the dependence of amnion formation on the presence of the head-fold. They possess other definite lesions, the effects of which will be described in another paper.

Condition of the Allantois in Anamniotic Embryos.

The allantois is well formed in four of these embryos; one (No. 57) was too young to show it externally. It is obvious that in the absence of the amnion the growth of the allantois

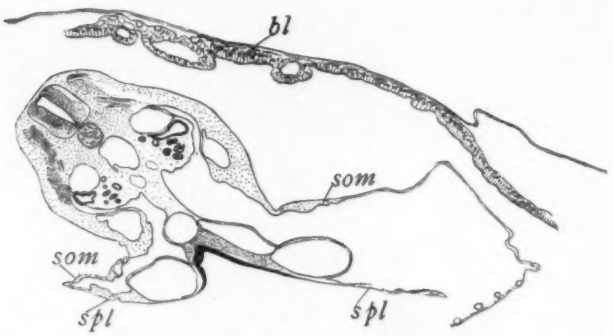


FIG. 16. Experiment 60. Section along the line *A-B*, Fig. 15. *bl.*, blastoderm overlying the embryo; *som*, somatopleure; *spl.*, splanchnopleure. On the right side there is a break in the continuity of somatopleure and splanchnopleure; this was evidently produced in the preparation, as the continuity is perfect some distance in front, and also behind.

must be attended with difficulties. When the amnion is normally formed a large free space is created above and around it, into which the allantois can freely spread. The absence of this space causes compression of the allantois, and changes the direction of its growth, but I do not think that the latter is much impeded. The mechanical force of the expansion of the allantois causes separation of the somatopleure and splanchnopleure to proceed more rapidly in its immediate vicinity than elsewhere (see Fig. 15). In experiment 124 the greater diameter of the allantois exceeds the greatest length of the embryo. I see no reason why this process might not provide all necessary space for its expansion. It might be, however, that the resistance offered would tend to cause accumulation of the products of excretion in the body of the embryo, and thus gradually poison it.

2. *Experiments on the Tail-Fold of the Amnion.*

I have also made a number of experiments on destruction of the tail-fold of the amnion. The results are in most cases com-

plicated by conditions that do not properly belong to the subject of this paper. There is but one uncomplicated case (exp. 18). In this experiment the hind-end of the embryo was cauterized immediately after the appearance of the tail-bud (Fig. 17), thus

FIG. 17. Experiment 18, operation diagram. Outline of embryo of chick of about 52 hours, after Duval. The ruled area represents the part destroyed by the heated needle.

destroying the tail-fold of the amnion. When the egg was re-opened forty-eight hours later, a well-developed embryo of about five days was found in which the amnion ceased with a free edge immediately in front of the hind-limbs (Fig. 18).

The conditions of the membranes in this embryo are otherwise very complicated and difficult to understand. Thus there is in addition to the amnion a fold of the blastoderm surrounding both amnion and embryo (Fig. 18). In the posterior half of the embryo the body-wall is directly continuous with the wall of the intestine as in 60. As this embryo will come up for description elsewhere, I shall not dwell further on this topic.

The fact that stands out distinctly is that the tail-fold of the amnion has not regenerated and that the head-fold has not compensated for the absence of the tail-fold by continuing its growth backwards. However, I have a number of embryos in

which a complete amnion has been found without any tail-fold. These embryos, are, however, defective at the hind end, so that

FIG. 18. Experiment 18. The embryo 48 hours after the operation. The tail-fold of the amnion has not regenerated. The amnion ends with a free edge in front of the hind-limbs. A fold of blastoderm is wrapped around the embryo and amnion. Under surface of blastoderm.

one has not to attribute any work of supererogation to the anterior lateral folds to explain the complete closure. This also will be discussed elsewhere.

GENERAL DISCUSSION.

The formation of the amnion of the chick seems to be a process with extraordinarily slight power of regulation.[1] A slight injury to part of its early rudiment sets the whole process astray. It is thus an extremely good example of correlative differentia-

[1] Barfurth ('02) notes incidentally in one of his experiments "die Amnion war regenerirt." As I understand him, he means by this simply that an aperture made in the amnion in the course of an experiment on the eye closed up. I can confirm this from my own observations. I have found that even considerable tears made in the amnion *after its formation* may close completely.

tion. The correlations in the development of the amnion are of three kinds:

1. *Mechanical.* — Under this head I class the elevation of the lateral amniotic folds, which takes place only after the establishment of the head-fold, and which is omitted, if for any reason the head-fold fails to appear or is destroyed.

2. *Trophic Stimulation.* — Under this head I class the influence of the traction exerted by the union of the right and left amniotic folds on the amniogenous somatopleure, and the influence of the turning of the embryo on the amniogenous somatopleure of the left side. The influence of the traction in either case is to increase the extent of the amniogenous somatopleure, in part (presumably) by stimulating its growth, in part undoubtedly by mere stretching. If, owing to failure of formation of the head-fold, such traction is not exerted on the somatopleure it does not expand nearly to the normal extent.

3. *Differential Stimulation.* — Under this head I class (doubtfully) the propagation of the ectamniotic thickening along the somatopleure; though this may be a process of self-differentiation.

Self-differentiation of the Formation of the Amnion. — The formation of the original rudiments of the ectamnion may be a process of self-differentiation, though the definite relation of the anterior and posterior rudiments to the head and tail respectively suggests correlation with their formation. Moreover, a slight histological differentiation appears between the amniogenous and choriogenous somatopleure, before, and even in the absence of, the formation of folds, which is apparently not correlated with any other of the processes observed.

Beyond this mere classification I do not desire to go at present, but will reserve a general discussion of principles until the completion of other parts of the present series.

In conclusion I simply summarize the results:

I. *Morphological.*

1. The amnion is primarily an organ of the ectoderm in the chick. The ectamnion first forms in front of the head and differentiates progressively backwards towards the posterior end,

where it is met by the posterior ectamnion differentiating forwards. Thus the amniotic zone of the somatopleure is marked off from the chorionic zone.

2. The head-fold is formed from the ectamnion with the coöperation of the amnio-cardiac vesicles and of the proamnion which is depressed between the former. The immediate prolongation of the head-fold is produced by the progressive fusion of the ectamniotic rudiments backwards, and it includes only an extremely small part of the proamnion.

3. The tail-fold is likewise formed primarily by the ectamnion with participation of the amnio-allantoic enlargements of the body-cavity.

4. There are certain constant secondary folds in the upper (right) limb of the amnion produced by the turning of the embryo. These persist longest in the region of the heart and immediately behind it.

II. *Experimental.*

1. Destruction of the anterior ectamniotic rudiment of one side prior to the formation of the head-fold of the amnion results (*a*) in permanent absence of the amnion back to the hind-limbs (exp. 57); (*b*) in inhibition of the growth, and almost complete suppression of the folds of the amniogenous somatopleure of the uninjured side; from which we may conclude—

2. That the growth of the amniogenous somatopleure is normally induced by the traction exerted on it by the progressive fusion of the folds, and that the uprising of the folds is due to the lifting power of the same process of fusion.

3. The tail-fold and posterior lateral folds cannot replace the anterior lateral and head-folds, nor can the latter replace the former.

4. Not only the initiation, but also the progress of the formation of the anterior lateral folds is dependent upon the perfection of the head-fold (exp. 36).

5. The absence of the amnion has, at least for a time, only a limited effect on the development of the allantois.

6. Inasmuch as the embryo may develop perfectly normally to the stage of five or six days without the amnion, it is obvious that the functional significance of the latter must be slight during

this period. It yet remains to be determined how far the embryo may develop without the amnion (see quotation from Dareste below).

7. There is a certain relation of interdependence between the formation of the amnion and the body-wall. In the absence of normal formation of the lateral folds of the amnion the closure of the somatopleure to form the body-wall proceeds more slowly than usual.

Dareste ('79) has observed total absence of the amnion in embryos of the chick. The condition was not, however, produced experimentally. His observations and conclusions are given in the following quotations:

"J'ai signalé, depuis longtemps, l'arrêt de développement de l'amnios et les anomalies nombreuses que cet arrêt partiel détermine chez l'embryon. C'est la cause la plus fréquente des monstruosités simples. Il y a des cas, beaucoup moins nombreux, il est vrai, dans lesquels l'amnios fait complétement défaut. L'embryon est alors en continuité directe, par son enveloppe cutanée, avec le feuillet séreux du blastoderme, qui ne s'est pas plissé pour former la poche amniotique. J'ai vu, dans plusieurs de ces cas, l'embryon se constituer d'une manière parfaitement normale. La paroi thoraco-abdominale s'était complétement formée, et la continuité de l'embryon avec le feuillet séreux constituait une sorte de cordon ombilical. L'allantoïde sortant de l'abdomen par se cordon s'était engagé entre le feuillet séreux et le feuillet vasculaire."

"Les embryons, ainsi privés d'amnios, peuvent vivre pendant un temps assez long. J'ai constaté l'absence compléte de l'amnios sur en embryon de treize jours, qui était plein de vie et parfaitement normal. Rien ne pouvait faire penser qu'il mourrait prochainement. Il est très-probable cependant qu'il n'aurait pas atteint l'époque de l'éclosion. L'absence de l'amnios aurait mis obstacle au développement complet de l'allantoïde: ce qui aurait produit l'asphyxie de l'embryon, comme je l'ai montré depuis longtemps. Le plus ordinairement l'absence de l'amnios amène la mort précoce de l'embryon. Souvent aussi elle détermine, dans son organisation, des modifications tératogéniques profondes."

"Toutes des observations nous font connaître le rôle physiologique de l'amnios dans la vie embryonnaire. Il est bien évident que l'amnios protége l'embryon contre toutes les actions mécaniques qui tendraient á le comprimer."

HULL ZOÖLOGICAL LABORATORY, UNIVERSITY OF CHICAGO,
April, 1903.

POSTSCRIPT.

After the foregoing paper was fully printed, my attention was called to an article by Weldon in which anamniote embryos of the fowl were described, and which I had overlooked owing to the fact that the observations were included in an article entitled "Prof. de Vries on the Origin of Species" (*Biometrika*, Vol. I., Part III., April, 1902). Partial or complete suppression of the amnion resulted from experiments to replace the water lost by evaporation in the incubator without preventing the process of evaporation itself. "A hole was made in the broad end of the egg-shell and the subjacent membranes, into which one end of a siphon, filled with water, was fitted. The other end of the siphon was placed in a reservoir of water, and the whole apparatus placed in an incubator. In from 20 to 30 per cent. of the embryos treated in this way the amnion was largely or entirely absent after incubation for three or four days."

Weldon does not discuss the mechanics of formation of the amnion, but treats the result simply as an example of a definite relation between the environment and an extremely stable character. Apparently the immediately effective factor in the experiments was the increased pressure within the shell, which, presumably, forced the embryonic area into immediate contact with the shell membrane, and thus prevented the uprising of the amniotic folds.

F. R. L.

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CROSSOBOTHRIUM LACINIATUM AND DEVELOPMENTAL STIMULI IN THE CESTODA.

W. C. CURTIS.

In the spiral valve of the "sand shark" (*Carcharias littoralis*) taken from the Woods Holl region there is found in a large majority of the specimens examined the Cestode, *Crossobothrium laciniatum*. This genus and species was first described by Linton ("Rept. U. S. F. Com." for 1886), and in subsequent papers appearing in the same publication or in the "U. S. F. C. Bulletin," he has added further important notes, the whole making an accurate and satisfactory systematic description.

A striking feature of the species is the remarkable clearness with which the important features of Cestode structure can be demonstrated. The water vascular system, main trunks and flame-cells can be seen in the fresh specimen with the greatest ease. Almost every detail of the complicated reproductive organs is seen in well-stained whole mounts of the motile proglottids and much of this in specimens freshly prepared. The mode of using the suckers on the head, the activities of the motile proglottids and their mode of egg-laying and the development of these eggs in sea-water as far as the six-hooked embryo are all easily demonstrated. Moreover, there occurs in the cystic duct of the squeteague (*Cynoscion regalis*), a not uncommon food of the "sand shark," a tetrabothrian larva which, if not the larva of *Crassobothrium laciniatum*, probably belongs to some very closely related form. This larva, which was first described and figured by Linton ("Rept. U. S. F. Com.," 1886), is again an extremely favorable object for study.

If it is possible to obtain conclusive evidence that this tetrabothrian larva of the squeteague is indeed the larva of *C. laciniatum*, we shall have but one gap in the life history of this species, viz., the transfer to the squeteague of the six-hooked embryo which develops in the open ocean.

Such favorable material it seemed to me might present, upon careful examination, facts which would be suggestive along the

line of some of the general problems involved in Cestode parasitism and development in addition to the possible opportunity for fixing the life history of this particular form. With this in mind I have been collecting all the data bearing upon the life history and during the summer of 1902 I made the first of a series of experiments in infection which I hope to continue and which may lead to more precise knowledge concerning the identity of the larva found in the squeteague.

I wish in this paper to describe the important features in the structure of the motile proglottids, its egg-laying and other activities, to give some observations on the larva from the squeteague and to discuss the view point which my study of the development in this and other Cestodes has suggested to me.

The Motile Proglottids.

When an incision is made in the spiral valve of an infected "sand shark" the Cestode is frequently found in such abundance that, as the elongated bodies and the motile proglottids writhe about in the chyle, one often wonders how there can be enough nourishment left for the host. I can confirm Linton's record, of "sand sharks" taken at different times, that in the great majority of individuals there are literally hundreds of this parasite in the spiral-valve to the exclusion of all others. When the parasites are examined in sea-water the alternate protrusion and retraction of the bothria, as described by Linton, can be observed for hours. When a scolex is compressed on a slide the flame cells of the water vascular system can be observed for a considerable time before they succumb to the abnormal conditions.

The ripe proglottids which can be pulled from the long strobilæ or found loose in the intestine are very active and constantly changing their shape. A typical outline in a partially extended condition is represented in Fig. 1, and the fully elongated condition is represented on a smaller scale in Fig. 2. At the anterior tip I have found in preserved specimens minute projections which have the appearance of cilia (*c*), but which will probably prove upon examination in the living specimen to be minute spikes similar to the larger ones on the penis (*p*).

The four ear-like flaps at the posterior end which give the

strobila its characteristic appearance are frequently curled back and outward, giving the posterior end a quite different outline. In the living specimen I have frequently seen masses of sperm ejected from the penis, but my records of this do not mention

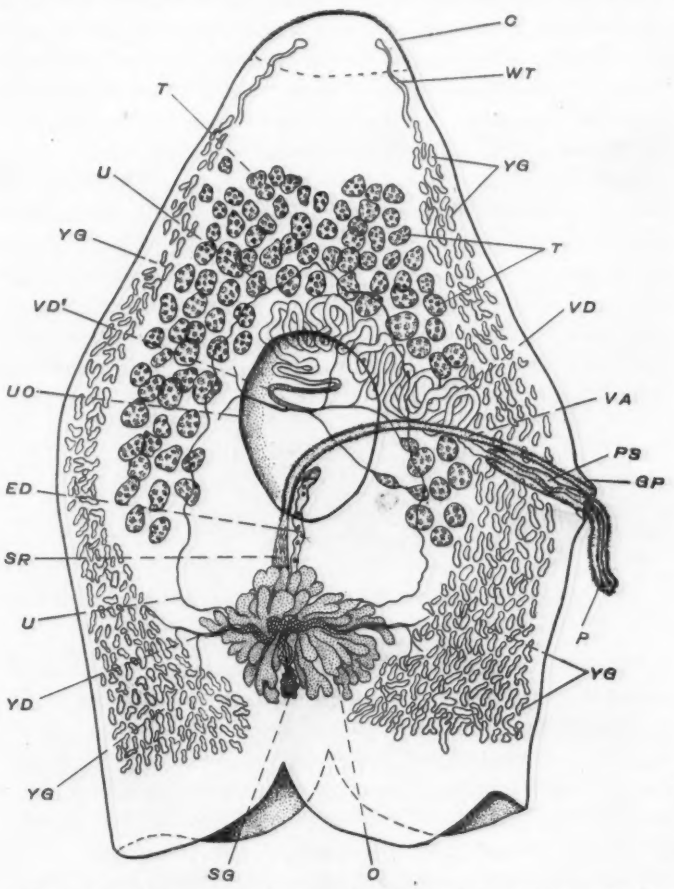

FIG. 1. Reproductive organs of a motile proglottid of *C. laciniatum*. *C*, cilia-like spikes at anterior tip; *ed*, egg duct from shell gland to uterus; *gp*, genital pore; *o*, ovary; *p*, penis; *ps*, penis sheath; *sg*, shell gland; *sr*, seminal receptacle; *t*, testes; *u*, uterus; *uo*, uterus opening through which the eggs escape; *vd*, vas deferens; *vd'*, denser inner end of same; *va*, vagina; *wt*, large water vascular tube; *yd*, yolk duct; *yg*, yolk glands.

the condition of the female organs in the proglottids thus observed. There are four main water vascular tubes. The larger pair lie on the same side of the body as the uterus opening and in the majority of cases one or both of them can be traced to a bulb-like enlargement on either side near the anterior tip of the proglottid. Posteriorly each one seems to end in the angle of the broadly wedge-shaped concavity formed by the projecting flaps. They here seem to end blindly against the cuticle which is perhaps perforated. There is no cross connection between these two vessels nor any common posterior opening such as is frequently stated to occur in *Tænias*. The second pair of main trunks are vessels of much smaller diameter and lie on the other side of the flat body immediately under the larger pair. Anteriorly they can sometimes be traced a little farther forward than the bulbs of the larger vessels, but do not seem to end in an enlargement. It is almost impossible to follow these smaller trunks for any distance posterior to the penis as the yolk glands are here closely packed together and obscure everything else.

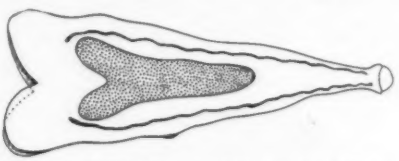

FIG. 2. Ripe motile proglottid fully extended, showing full uterus and the larger pair of water vascular trunks.

When the proglottids are examined alive much of their structure is obscured through the presence in the parenchyma of the highly refractive and closely packed granules of calcium carbonate. A very easy way of ridding the proglottid of this and preparing it for immediate examination is to use ten per cent. nitric acid and the pressure of a cover-glass. This dissolves the calcium carbonate and leaves the specimen quite transparent. This is a valuable method for the rapid examination of the principal organs, but for the finer details one of course needs more careful fixation and a good stain. I have found corrosive sublimate with about five per cent. acetic acid followed by Czokor's alum cochineal an excellent combination for the demonstration of the features given below.

The cirrus (*p*, Fig. 1) is eversible, working on the same principal as a Nemertean proboscis, a type common in Cestodes. From its base the much-coiled vas deferens (*vd*) leads away and is found throughout these coils crowded with sperm. At its inner end it has a denser wall and is of less diameter for a short distance (*vd*) and then divides into the vasa efferentia which can be seen radiating to the area in which the testes are located and in favorable cases followed down to the testicular follicles themselves (Fig. 1).

The vagina (*va*) which opens on the genital papilla just above the penis will be seen in the figure to pass inward and curve around backward, passing behind the mass of finger-like follicles which constitutes the ovary (*o*). It is here enlarged into the

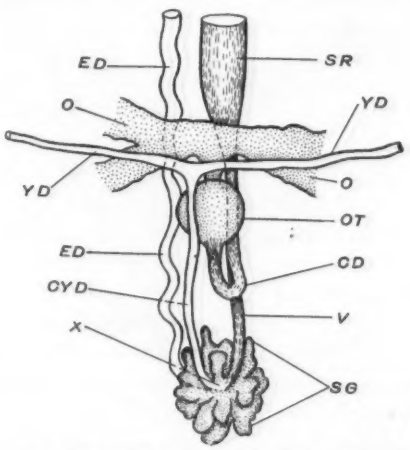

FIG. 3. Ducts of female complex with ovary lobes left out and ducts reflected slightly to show connections. *cd*, duct from oötype to inner end of vagina; *cyd*, common yolk duct; *ed*, egg duct; *o*, ovary; *ot*, oötype; *sg*, shell gland; *sr*, seminal receptacle of vagina; *v*, inner end of vagina; *x*, meeting place of ova and yolk; *yd*, right and left yolk ducts.

seminal receptacle which will be found full of sperms. Lying among the posterior lobes of the ovary is the shell gland (*sg*) to which the yolk is delivered from a common yolk duct formed by the union of a single yolk duct (*yd*) coming from either side. Extending anteriorly from the shell gland and beneath the ovary

in this figure is the egg duct (*ed*) which conveys the eggs to the uterus. The complex of ducts in this region is shown in a reconstruction from sections represented in Fig. 3. The lobes of the ovary which are packed closely around the ducts are here omitted. This figure may be compared with what is shown in Fig. 1, where some of the same parts appear. The lobes of the ovary all converge upon a right and left portion (Fig. 3, *o*) and these main parts, on uniting, open posteriorly into a spherical cavity (*ot*) with thick walls, which is probably where the ova and sperm meet. A duct (*cd*) passes from this cavity to the inner end of the seminal receptacle (*sr*) and thence straight back to the shell gland (*sg*). Into the shell gland the common yolk duct (*cyd*) opens and from this common meeting place of the yolk and fer-

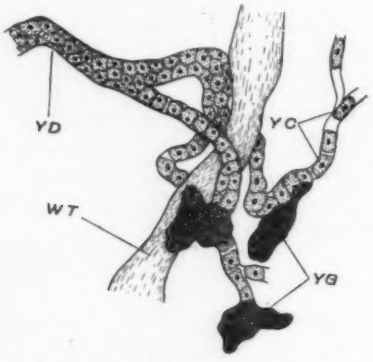

FIG. 4. Branching of yolk duct to yolk glands. *yd*, yolk duct; *yc*, yolk cells in duct; *yg*, yolk glands; *wt*, large water vascular tube.

tilized ova, after the acquisition of a shell, the fully formed egg passes up the egg duct (*ed*) into the uterus.

The right and left yolk ducts branch as they reach the areas of the yolk glands on either side and some of the branches may be seen going to individual yolk follicles (Fig. 4). These main branches are often found closely wrapped around the large water vascular tubes (*wt*) of either side. Yolk cells (*yc*) may often be found on their way down these ducts and accumulated in large numbers at their median ends. They are also seen in the short ducts which run from one yolk gland to another in all parts of

the mass. The yolk-producing organ consists of follicles densely packed with yolk cells and distributed in the proglottid as the figure indicates (*yg*, Fig. 1).

Fig. 1 represents a specimen killed under pressure and in which the uterus had been ruptured and the eggs squeezed from the oval hole represented by the dark outline in the center of the proglottid. Very much the same sort of a hole is left when the proglottid ruptures itself at this point in the normal egg-laying. The extent of the uterus cavity is indicated by the outline (*u*) in the figure. The condition of the intact uterus and the place of its rupture will be explained in describing the egg-laying of the ripe proglottids.

Activities of the Motile Proglottids.

The proglottids of *Crossobothrium laciniatum* are an extreme case of what is usually termed the "motile" condition. So definite are their movements and activities that one is constantly thinking of them as though they were individual animals of a species entirely distinct from the parent scolex. When observed in the chyle they are seen writhing about, contracting and elongating rhythmically and bending their bodies into an arch along the axis of breadth, now one way, now another. If we measure the maturity of a proglottid by its size and the number of eggs accumulated in the uterus the conclusion is reached that the proglottids as taken from the spiral-valve are of diverse ages, for one finds a considerable variation in the number of eggs accumulated in the uterus and a correlated variation in the size of the proglottids.

When placed in clean sea-water the smaller proglottids do not lay their eggs, while the large ripe ones will almost immediately do so. These facts seem to indicate that the proglottids may be shed off from the strobila some time before they are ripe and remain in the shark's intestine until they are fully loaded with eggs and ready for the laying. The enormous number of proglottids usually found in a single spiral-valve is another fact in favor of this conclusion. On the other hand fully mature proglottids are frequently found on the end of a strobila (Fig. 5), showing that they may mature while still attached to the parent stock.

When carefully examined the ripe proglottids at the posterior end of a strobila (Fig. 5) or the mature motile proglottids found free in the chyle show a breast-like protuberance upon that face on which the uterus opens. The resemblance of this to a breast is heightened by the existence of a nipple-like prominence at the summit, as is shown in the side view given in Fig. 5. The general protuberance is caused by the distension of the uterus, though it sometimes seems to be enhanced by a concavity on the opposite face of the segment as the dotted line of the figure indicates. Motile proglottids when in this ripe condition show, if examined in the chyle, the ordinary writhings and indefinite locomotor movements noted above. If, however, a number of these ripe and full proglottids are transferred from the chyle into clean sea-

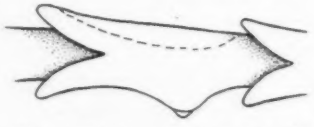

FIG. 5. Side view of a ripe proglottid.

water the egg-laying will presently be observed. In making observations on this process I was accustomed to select carefully ten or a dozen proglottids which seemed fully ripe and transfer them all together into a dish of clean sea-water. When this was done it was found that about eight out of ten thus selected laid their eggs in three or four minutes. Any of those remaining might lay after a little longer period or not at all. A similar reaction of whole chains of proglottids is recorded by Schauinsland (Jena, Zeitsch. 1886), for *Bothriocephalus latus*, *Trianophorus nodulosus* and *Ligula simplissima*.

When proglottids of *Crossobothrium* are taken at random and thus placed in sea-water only a small proportion, no more than one fourth or one fifth, will ever lay their eggs. When the small proglottids which have only a few eggs in the uterus are thus taken no egg-laying follows in any case.

That the proglottids as found in the spiral-valve at any one time are not all of same maturity is thus clearly shown and I think we are justified in the conclusion that these immature proglottids tend to remain in the spiral-valve until they become fully ripe

and then to pass out with the fæces, even though their early detachment from its strobila may have been premature and caused by the outward passage of the excreta or the contraction of the intestine.

The manner in which the egg-laying proceeds in any single proglottid thus placed in sea-water is a very interesting thing to watch. Extreme writhing movements of a quite definite sort begin at once. The proglottid bends along its axis of breadth until it is almost a closed ring, the pointed anterior end sometimes passing into the angle made by the posterior flaps (Fig. 2) and thus reminding one of an acrobat who could bend backward until his head should be thrust between his legs from behind, then the proglottid straightens and the bend is reversed, it

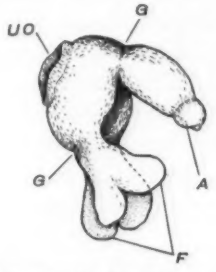

FIG. 6. Proglottid in the act of egg-laying seen from the side. Lettering same as for Fig. 7.

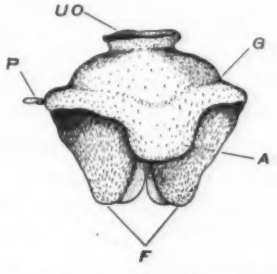

FIG. 7. Proglottid at close of egg-laying seen from anterior end. *a*, anterior end; *p*, penis; *g*, groove marking outline of uterus; *uo*, ruptured area through which the eggs escape.

straightens again and bends into the first position and so on; these motions continue until the nipple-like prominence of the protruding body bursts and the liberated eggs gush forth. When the break occurs the extreme violence of the writhing ceases and the proglottid bends backward rather more than forward (Figs. 6 and 7) until all the eggs have been expelled, when a gaping hole is presented (Figs. 6 and 7) where once was the distended uterus. Even when there is hardly an egg left within it, the straining movements of the proglottid continue as though it were making sure that not a single egg remained. Figs. 6 and 7 represent proglottids in which the egg-laying had been almost

accomplished and show the characteristic attitude of the proglottid from a front and side view.

Proglottids which have thus stripped themselves of their eggs may continue to live in sea-water for a day or two, but I have not experimented with them to ascertain how long their existence may be prolonged.

The rupture of the uterus may be very readily produced in a proglottid having any considerable accumulation of eggs, if a little pressure is applied with a cover-glass or otherwise. But the process above outlined is something brought about spontaneously by the proglottid itself after it is transferred to sea-water. Proglottids which have been artificially compressed in killing, for whole preparations almost always have the uterus ruptured and the eggs discharged. They then present the appearance shown in Fig. 1, of a large oval hole opening into the uterus cavity, while the boundaries of the latter can be traced as a very delicate outline still conforming to the general outline of the full uterus. A proglottid which is in the last stages of egg-laying after the spontaneous rupture of its uterus shows the same sort of opening, but perhaps more widely distended. Such specimens which were killed without compression are shown in Figs. 6 and 7.

My observations are then that the proglottids when large and having the uterus full of eggs (Figs. 2 and 5) will, by a quite definite series of muscular contractions and writhings, rupture the nipple-like prominence at the summit of the protruding uterus (Fig. 5) and allow the eggs to gush forth, the proglottid continuing its writhing movements in a less pronounced degree even after all the eggs have been shed (Figs. 6 and 7). The fact that this egg-laying occurs immediately after the ripe proglottid is transferred from the chyle to clean sea-water will, I think, convince any one that the same process occurs when a ripe proglottid of *Crossobothrium* passes in the normal course of its existence out of the shark's cloaca into the water of the ocean. We may conclude I think that such a proglottid, upon coming into contact with the sea-water outside, goes through muscular contractions similar to those observed in the laboratory and lays its eggs free in the open ocean, and that these pelagic eggs are thus widely

scattered. The short period between the first contact with the outer water and the egg-laying indicates that the infection of the intermediate host is, by means of countless embryos, developed in the open ocean and not by the eating of the intact proglottids with their contained eggs.

Egg Development.

On collecting the eggs laid by proglottids in the laboratory and placing them in dishes in which the sea-water can be kept reasonably pure, development ensues as far as the six-hooked embryo stage which I have represented in Fig. 8, drawn from the living specimen. I did not succeed in obtaining embryos beyond this stage, and therefore cannot say whether the embryo

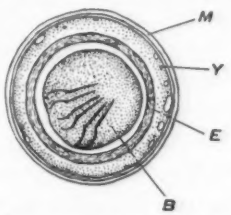

Fig. 8. Six-hooked embryo of *Crossobothrium laciniatum*. *m*, egg membrane; *v*, remains of outer envelop; *e*, ectoderm of Schaunisland; *b*, six-hooked embryo.

enters the next host in this condition or as a ciliated larva (Schauinsland, "Bothriocephalidæ," '86) which subsequently hatches from the embryo figured.

In the common Tænias and those Cestodes which have similar hosts and conditions of life-history, the fertilized eggs on passing into the uterus develop *there* into six-hooked embryos and remain in that stage until they reach the tissues of the intermediate host. In the Bothriocephalidæ, Schauinsland describes *B. rugosus* as having such an intra-uterine development as far as the six-hooked embryo, and *B. latus*, *Trianophorus nodulosus* and *Ligula simplissima* as producing eggs which develop only when they reach the water. In the last three species the eggs accumulate in the uterus in the condition of resting oösperms surrounded by their yolk and the egg-shell, but do not develop until they are laid in the water by the parent proglottid after this has left the host's intestines.

My material was prepared for the general anatomy rather than for this particular point, and the egg capsules are greatly shrunken, but as nearly as I can make out from a careful examination of the uterine eggs they are all in the condition above noted, viz., a resting fertilized ovum plus the yolk cells and egg capsule. In Cestodes having this mode of development, therefore, the eggs accumulate in the uterus, but do not develop until they are laid by the proglottid. The resting stage is comparable to the resting condition known in many forms which lay winter eggs or eggs which develop only after a considerable time. In any given proglottid such Cestode eggs are of diverse ages, depending upon how long they have been in the uterus, but are all alike inhibited from development until the proper conditions are present. Upon contact with the external water this resting stage is stimulated, or, we might say, some inhibition is removed, and development ensues, continuing as far as the six-hooked embryo. Whether there is some specific thing in the sea-water which can be fixed upon as the stimulus to development I have not ascertained, but it has seemed to me quite possible that the stimulus can be located in some specific chemical constituent of the sea-water.

From what we know of other Cestodes it is unlikely that the six-hooked embryo of *Crossobothrium* can develop further in the water, and that if this embryo does not find the appropriate stimulus for further development by meeting with its next host it must perish. What this next host is one would perhaps discover as much by accident as by the most persistent work. Could I clearly demonstrate that the larva found in the squeteague is the young of *Crossobothrium laciniatum*—and there is a good deal of general evidence that this is the case—I think that the nature of the squeteague's food (young herring, adult herring, menhaden, etc.—Peck, "Sources of Marine Food," U. S. F. C. Bull., '95) would lead us to suspect that the six-hooked embryo, instead of passing directly to the squeteague, might have an intermediate host like the menhaden, herring, or some fish which feeds upon the microscopic elements in the sea-water.

Developmental Stimuli in the Cestoda.

To one examining closely the present trend of opinion regarding the process which our nomenclature still designates as fertilization it is, I think, quite apparent that the evidence and conclusions so logically and convincingly set forth by R. Hertwig[1] are gaining a wide acceptance both among special workers and among those who are viewing the data with a somewhat broader perspective. The details of Hertwig's paper had been already accepted by some of the investigators whom he cites, and so far as they concern the Protozoa they are mentioned by Calkins in his recent book as well-established facts, but this cannot detract from the able manner in which Hertwig has summarized these facts and indicated the important conclusions to be drawn from them. The work which to most persons offers convincing evidence of the twofold nature of the process we have been calling normal fertilization is the work in "artificial parthenogenesis" initiated by Loeb and Morgan. One could hardly ask for a more convincing proof that the union of the germ plasms and the "developmental stimulus," as Hertwig calls it, are distinct and separable phenomena although they have become almost indissolubly connected with one another throughout the Metazoa.

It having been shown in these experiments so far as they have now gone that a stimulus to development may be operative entirely independent of any union of the germ-plasms, as for example in the development of eggs of echinoderms or worms upon treatment with salt solutions and other stimuli, we should I think seek no less for the converse proposition, viz., *the union of the germ-plasm and the absence of developmental stimulus* (as evidenced by the absence of development) *while the oösperm continues its life.* We should then seek for the proper stimulus to this resting condition and by means of this stimulus initiate at will the developmental changes. To illustrate by a hypothetical case, suppose we could have a form where it were possible to bring about at will the union of the ovum and spermatozoön and their nuclei but by subtracting

[1] "Mit welchen Recht unterscheidet man geschlechtliche und ungeschlechtliche Fortpflanzung?" *Sitz. Ber. Gesel. f. Morph. und Physiologie in München*, Nov., 1899. Translated in *Science*, N. S., Vol. XII., no. 312, Dec. 21, 1900.

the " developmental stimulus " to have the resulting oösperm go into a resting state which would result eventually in its death unless the " stimulus to development " intervened to start a new cycle. If this developmental stimulus, or some substitute for it, and its action were accurately known we might apply it at will at any time after the union of the germ-plasms was completed, and so long as the oösperm remained alive it would respond by beginning the new cycle of development.

In the thorough understanding of the two apparently distinct processes involved in fertilization as hitherto understood we should I think be greatly aided by the investigation of the problem just outlined which is the *converse* of that brought out by the work on " artificial parthenogenesis."

We have, it seems to me, cases of normal development which are parallel to this converse proposition in just the same way that normal parthenogenesis is a parallel to the artificial development induced by salt solutions and other stimuli. These cases are seen in the development of those forms in which the fertilized egg has a long resting stage. Where freezing or desiccation is necessary for such subsequent development, this condition may be regarded as one of the developmental stimuli, although if one tries to picture how a state of affairs necessitating this particular condition could have arisen in the past he must, I think, feel certain that though extreme cold or dryness may now be a necessary factor it must originally have been unnecessary if not a thing fatal to the further existence of the organism.

Any one familiar with biological literature can readily recall numerous cases of eggs with longer or shorter resting periods following upon the union of the germ plasms. The particular case I call attention to is that presented by those Cestoda which in their development have *resting fertilized eggs* gradually accumulating in the uterus as in the case in *Bothriocephalus latus*, *Trianophorus nodulosus*, *Ligula simplissima*, etc., and in *Crossobothrium laciniatum*. On referring to the description which has been given of the production of the eggs and their extra-uterine development it will be seen that this illustrates particularly well what I have termed the converse of artificial parthenogenesis and that the hypothetical case which I set forth on page 137 might be substi-

tuted almost word for word for a description of what occurs in one of these Cestodes.

In addition to the phenomenon of a resting stage in their early development the Cestodes just mentioned, and indeed all other Cestoda, present in their subsequent life history a feature which I have found interesting when considered in connection with the primary "developmental stimulus" which starts the oösperm upon its course. Such a consideration of the subsequent facts of Cestode life history may perhaps widen our conceptions regarding the nature of one of the two phenomena which exist side by side in normal fertilization. How this is will be easily apparent if we recall the life history of *Crossobothrium* or any Cestode having a similar extra-uterine development.

The female reproductive organs of each proglottid produce ova which on being fertilized become surrounded by their yolk supply and encased in a tough shell. Without undergoing any developmental changes they accumulate in the uterus where they remain in this condition until the time of egg-laying. They are thus of very diverse ages if we date the age of each from the time of the entrance of the spermatozoön, but all are in the same *resting unicellular state*. We have here the union of the germ plasms, but the stimulus to development delayed for a period which is long or short, depending upon the age of the individual oösperm. The stimulus to development is normally found in the contact with the outside sea-water when the eggs are shed, for the cleavage begins only when they are thus set free. Development proceeds so far as the six-hooked embryo stage when death ensues unless the proper host is found. In the case of *Crossobothrium* there is perhaps a primary intermediate host between the six-hooked embryo and the squeteague in which case the six-hooked embryo which infects this intermediate host receives a stimulus to development which sends it so far as the resting stage which is attained in that particular host, and here it stops and eventually comes to naught unless it is carried into the next host, the squeteague, where it finds a new stimulus to further developmental changes and attains in the cystic duct of this fish its development to the full structure of a tetrabothrian larva.

Here again death ensues unless the next stimulus in the series is forthcoming, viz., the contact with the digestive juices of the "sand shark's" stomach. When this stimulus is furnished the tetrabothrian surviving the wreck of its teleost host develops into the final adult condition.

The foregoing is stated in sufficiently general terms to be applicable, *mutatis mutandis*, to any Cestode having an extra-uterine development and whether or not the life history outlined for *Crossobothrium* is the correct one does not affect the general conception of Cestode life history which I am attempting to portray.

In stating the above I have spoken of the reaction of the embryo of a given stage to its particular stimulus just as I spoke of the reaction of the oösperm to the stimulus which initiates the whole cycle. If we ask ourselves what is the essential nature of the reaction of the oösperm to the delayed developmental stimulus we must designate it as primarily a reaction manifest in so many cell divisions and subsequent differentiations. In what does the result produced by any one of the stimuli, which, if the embryo runs its whole course, become applied to each of the successive stages, differ from the result produced by the stimulus which acts upon the oösperm? Cannot the result in each instance be formulated in the same way, viz., that the stimulus causes cell divisions and subsequent differentiation? And should we not speak of all of them as "developmental stimuli"?

The exact nature of the stimulus at each stage is a thing which in spite of many technical difficulties would be open to investigation and something which we may hope eventually to understand, but however diverse the stimuli might be we should still have, as above stated, changes of the same nature resulting from each successive stimulus. The stimulus which is in the first instance something in connection with the sea-water is probably in the other cases a change in nourishment incident to the change of hosts, but in every case the *result* of the stimulus may be stated in the same general terms, viz., cell division and subsequent differentiation which ends in a condition of stable equilibrium in which the animal finally perishes unless the next stimulus is forthcoming.

Viewing then the life history of such a Cestode from this point of view we have first the union of the germ plasms followed by a resting stage of varying duration. A stimulus furnished by the contact with the sea-water when the eggs are laid brings about the changes resulting in the six-hooked embryo. This embryo when it receives a certain stimulus (condition of nourishment or otherwise) from the intermediate host goes as far along the course of development as the mid-larval stage and stops again. On reaching the stomach of the final host the last stimulus of the series is furnished and the adult condition attained.

In those Cestoda which have an intra-uterine development, *i. e.*, forms in which a six-hooked embryo develops in the uterus, we find the primary developmental stimulus intimately associated with the fusion of the germ plasms as in the fertilization of most Metazoa, though in *Bothriocephalus rugosus* (Schauinsland, '86) the intra-uterine development may not begin for several weeks after the eggs have begun to pass into the uterus cavity. In such cases the comparison between the primary "developmental stimulus" and the developmental stimuli which follow is of course not so patent, although it is none the less legitimate. Whether what I have called a "*developmental stimulus*" in these several cases shall be found eventually to be some new condition which the egg or embryo meets or be found to be the removal of some existing condition which has been inhibiting the development is of no consequence here since the removal of an inhibition may be spoken of as a stimulus, and since the important thing is not the nature of the stimulus but the similar reaction of the animal in each case.

In conclusion I may say that no facts not already familiar to students of Cestode life history have been set forth in the section of this paper just concluded, nor can it be claimed that the apparently two-fold nature of fertilization has not been in recent years more than once promulgated. The comparison between the reaction of the oösperm to the primary developmental stimulus and the reaction of the larval stages each to its special stimulus has interested me and it has seemed to me worth while to attempt the formulation of Cestode life history from this point of view. I also believe that the two-fold nature of fertilization

has not yet reached such a point of general acceptance or rejection, but that it will bear further illustration in any case in which there are facts particularly germane to the question and with these two points in view I have attempted the foregoing formulation.

UNIVERSITY OF MISSOURI,
COLUMBIA, MO., May 1, 1903.